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<p>(54) Title: MICROSATELLITE MARKERS FOR IDENTIFYING CANINE GENETIC DISEASES OR TRAITS</p> <div data-bbox="381 1165 1274 1501" style="text-align: center;"> </div> <p>(57) Abstract</p> <p>Microsatellite markers are provided which are useful in identifying linked markers for canine genetic diseases and traits. The microsatellite markers are derived from regions of genomic DNA which contain a repeat motif, flanked by unique sequences. The number of units contained within the repeat motif is variable, such that various different alleles are present in any given population. The microsatellite markers and their progeny are especially useful in detecting genetic diseases not phenotypically visible and identifying carriers of recessive diseases, as illustrated in the figure. In a preferred embodiment, microsatellite markers are provided which may be used to detect the canine copper toxicosis gene.</p>		

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MICROSATELLITE MARKERS FOR IDENTIFYING CANINE GENETIC DISEASES OR TRAITS

FIELD OF THE INVENTION

This invention relates generally to genetic markers and methods of making
5 and using such markers, and more particularly, to a microsatellite marker that may
be used to detect copper toxicosis in canines.

BACKGROUND OF THE INVENTION

Due to inbreeding and the relatively shallow gene pool, a large number of
genetic diseases are present in dogs (Clark, R.D. et al., *Medical and Genetic*
10 *Aspects of Purebred Dogs* (Forum Publications, Fairway, KS) (1994) and Robinson,
R., *Canine Pract.* 16:29-34 (1991)). Some of these genetic diseases such as copper
toxicosis in the Bedlington terrier breed, are so prevalent in a particular breed that
the mutant allele frequency may be higher than that of the normal allele (Herrtage,
M.E. et al., *J. Small Anim.* 28:1141-1151 (1987); and Yuzbasiyan-Gurkan, V. et al.,
15 *Genomics* 15:86-90 (1993)). Other genetic diseases cross many breeds, as
exemplified by progressive retinal atrophy causing blindness (Barnett, K.C., *Adv. Vet.*
Sci. Comp. Med. 20:9-67 (1976)) and hip dysplasia resulting in painful and crippling
arthritis (Corley, E.A., *Small Anim. Pract.* 22:570-593 (1992)).

Canine copper toxicosis (CT) is an autosomal recessive genetic disorder of
20 copper accumulation which results in severe liver damage. Unless specific anti-
copper treatment is instituted, affected dogs die by three to seven years of age.
While reported in several breeds, it is best characterized in Bedlington terriers, with
the frequency of the defective gene being estimated at 50%. The disease is also
prevalent in the West Highland White Terrier and Keeshond.

25 Currently, the only method for diagnosing affected CT dogs is by a
quantitative liver copper assay in a liver biopsy sample, after one year of age.
Unfortunately, heterozygous and homozygous normal animals are indistinguishable
from each other by this test. In order to determine if a dog is a heterozygous carrier,
test-breeding strategies must be employed which require that there be a dog of a
30 known genotype to breed against the potential carrier. This process is very costly
and results in the birth of many affected individuals. It is therefore impractical for
breeders to identify breeding stock free of the gene and currently carriers of the
gene are only identified after they are found to be the parents of an affected dog.

Because like CT, many of the canine genetic diseases are recessive, various
35 methods have been investigated which would identify, on a molecular level,
phenotypically normal carriers. One method that has been employed is the whole

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gene subtraction method. This approach requires the sorting out of differences between DNA from those with or without the disease or trait with molecular manipulation methods. Unfortunately, this technique is somewhat impractical and requires that all variability within individuals with the trait as well as the variability within those without the trait independent of the trait, be differentiable from the one or few that are dependent on the trait. Furthermore, this method has only been demonstrated on very simple organisms such as yeast, and while this technique appears theoretically possible for higher species, it rapidly becomes impractical, as it requires many breeding studies of large numbers of affected animals.

10 An alternative method, the use of restriction fragment length polymorphisms (RFLP), is extremely labor intensive and expensive with respect to both characterization and analysis. Furthermore, this technique requires large quantities of DNA, generally is limited to only two alleles, and only a few loci have thus far been characterized for the canine genome. It appears that with this method, a separate genetic system must be generated for each breed of dog, and such a library may not be sufficiently variable in most situations of interest.

20 The randomly amplified DNA fragment length polymorphism (RAPD) approach uses random primers to amplify fragments of genomic DNA that vary from individual to individual within a species. While the primers are relatively easy to generate, the method is unreliable with minor experimental changes resulting in the resolution of different DNA band patterns. Furthermore, only a few such bands have been characterized for the canine genome.

25 The candidate gene method is another alternative wherein one or more candidate genes is identified based on what is known about the biochemical and clinical or other phenotypic attributes of the disease or trait and information about similar conditions in another species where a gene has been identified for a similar trait. This approach was taken in evaluating genes linked to the Wilson's disease gene in humans, a disease similar to CT. Unfortunately, the genes linked to the Wilson's disease in humans were not linked to CT in dog (Yusbasiyan-Gurkan, V. et al., *Genomics* 15:86-90 (1993)). Thus, even under the best-case scenario, the candidate gene method is merely a guess and the approach is of course, further limited by the availability of identified genes.

35 Because canine pedigrees for various genetic disease are abundant, with several generations and two or more affected members present in many cases, these pedigrees lend themselves to linkage studies, provided polymorphic markers

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are available. Since most of the breeding is controlled, identification of linked markers would allow concerned breeders to greatly reduce the incidence of these diseases in future generations.

One type of marker that has been developed consists of simple sequence length polymorphisms (SSLPs). SSLPs arise from a varying number of repeats of a simple sequence, such as a dinucleotide repeat at a given locus, and have been reported to be frequent in most eukaryotic genomes (Tautz, D. et al., *Nucleic Acids Res.* 12:4127-4138 (1984)). Such loci, also referred to as microsatellites (Tautz, D., *EXS: DNA Fingerprinting: State of the Science* 1:21-28 (1993)), are best exemplified by those containing the (CA)_n motif and are found to be highly polymorphic in many species and are being successfully used in the construction of genetic maps of the human (Weissenbach, J. et al., *Nature* 359:794-801 (1992)), mouse (Dietrich, W. et al., *Genetics* 131:423-477 (1992)), rat (Serikawa, T. et al., *Genetics* 131:701-721 (1992)) and bovine (Barendse, W. et al., *Nat. Genet.* 6:227-235 (1994)) genomes. High polymorphic information content and amenability to analysis by polymerase chain reaction (PCR) and thus to possible automation, make microsatellites excellent linkage and mapping tools.

CA microsatellites from the canine genome have been identified and their polymorphism evaluated on sets of unrelated dogs (Holmes, N.G. et al., *Anim. Genet.* 24:289-292 (1992)) or mixed bred dogs and beagles (Ostrander, E.A. et al., *Genomics* 16:207-213 (1993)). Presently there are about 150 SSLP-type markers for the canine genome available. Unfortunately, these known markers lack the ability to detect a linked marker for any genetic trait, because of the low probability of finding a linked marker sufficiently close to a given genetic locus, to ensure detection. Many purebred dog populations have a relatively high level of inbreeding which makes it important that such markers be very polymorphic. Further, important genetic diseases occur across many dozens of breeds, requiring the markers be polymorphic in most, if not all, breeds with many different breeds having varying sets of genetic problems.

It would thus be desirable to provide a method for identifying genetic diseases and traits in canines. It would also be desirable to provide a method for identifying genetic diseases and traits in canines which has high variability and low breed specificity. It would further be desirable to provide a method which allows breeders to select and breed for certain favorable characteristics, or conversely, to avoid unfavorable diseases and traits. It would further be desirable to provide a method

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which allows the detection and screening of a recessive genetic disease such as copper toxicosis, which is phenotypically undetectable in heterozygote carriers. It would further be desirable to provide a method for identifying a carrier of a genetic disease or trait and affected individuals without undergoing test-breeding experiments. It would also be desirable to provide genetic markers for the canine genome. It would further be desirable to provide a marker for the CT gene in canines.

SUMMARY OF THE INVENTION

A set of microsatellite markers are provided which are useful in identifying linked markers for canine genetic diseases and traits. In particular, five hundred and nineteen microsatellite DNA markers are provided which are highly variable within and across many breeds of dogs. The microsatellite markers are derived from regions of genomic DNA which contain a repeated motif *e.g.*, (CA)_n, flanked by unique sequences. The number of units contained within the repeat motif is variable, such that various different alleles are present in any given population. The unique flanking sequences may be used as polymerase chain reaction (PCR) primers which allows for the rapid amplification and characterization of each locus from a small amount of DNA. Thus, each microsatellite marker has a unique set of primers. The microsatellite markers and their progeny are especially useful in detecting genetic diseases not phenotypically visible and identifying carriers of recessive diseases. In a preferred embodiment, microsatellite markers are provided which may be used to detect the canine copper toxicosis gene.

In addition to identifying canine genetic diseases such as copper toxicosis, the microsatellite markers may also be used to create a genetic map of the canine genome, generate specific breed profiles, settle parentage disputes and identify dogs by DNA fingerprinting. Pedigrees of affected individuals, their siblings, parent and progeny can also be created. Breeders and owners can thus choose breeding stock thereby reducing and possibly eliminating the incidence of specific genetic diseases.

Additional objects, advantages, and features of the present invention will become apparent from the following description and claims taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and by referencing the following drawings in which:

5 Figure 1A is a bar graph showing the average and standard deviation of heterozygosity percentages across loci within a breed;

 Figure 1B is a bar graph showing the average and standard deviation of heterozygosity percentages across breeds within a locus;

10 Figures 2A-2D are photographs of gels showing marker locus D02011 in various breeds; and

 Figure 3 is a photograph of a gel showing segregation of alleles at the C04107 locus in a Bedlington terrier pedigree.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 Five hundred and nineteen microsatellite markers from specific gene loci are provided which are highly variable within and across many breeds of dogs. The microsatellite markers of the present invention comprise a repeat motif e.g., (CA)_n, found in the canine genomic DNA, flanked by unique sequences. The unique sequences (also referred to herein as primer pairs) may be used as PCR primers, allowing the rapid amplification and thus detection of the sequence of interest in a
20 small DNA sample. Table 2A sets forth the microsatellite markers of the present invention. The microsatellite markers and their progeny are especially useful in detecting genetic diseases not phenotypically visible and identifying carriers of recessive diseases.

25 In a preferred embodiment, microsatellite markers are provided which may be used to detect a carrier of the canine copper toxicosis gene. As further set forth in Specific Example II below, marker locus C04107 may be used to predict the inheritance of alleles at the copper toxicosis locus. C04107 has also been used to locate two other marker loci C04107B and C04107C, which either singly, or as a group, may also be used to detect the copper toxicosis gene.

30 The method of the present invention is useful for identifying disease free individuals (homozygous normal), carriers (heterozygous) and affected individuals (homozygous affected) at any stage of development. While a single marker may fail to provide the required information in any particular pedigree, a series of progeny

markers will, and thus such a family of progeny markers derived from the linked markers set forth herein, are also included in the invention.

SPECIFIC EXAMPLE I

Materials and Methods

5 *Isolation and Characterization of Microsatellite Loci.* Established protocols were used for the cloning and screening procedures as described (Sambrook, J. et al., *Molecular Cloning. A Laboratory Manual* (2nd ed. Cold Springs Harbor: Cold Springs Harbor Laboratory Press) (1992)). Genomic DNA was isolated from a peripheral blood sample from a Labrador retriever and partially digested with *Bam*
10 *HI*. Size selected fragments purified from agarose gels using QIAEX beads (Qiagen Corp., Chatsworth, CA) were cloned into the phagemid vector pBS (Stratagene, La Jolla, CA) to construct a library of average insert size of 600 bps and propagated in the host XL-1 blue. The library was plated at low density (about 500 colonies/plate) without amplification. Duplicate nitrocellulose colony lifts were prepared, denatured
15 and hybridized with (CA)₁₆ oligomer, labeled with ³²P dCTP using terminal transferase. Positive colonies were picked with a sterile pipette tip and lysed in 50 μ l of a solution consisting of 1% Triton X 100, 20 mM Tris and 2 mM EDTA. Using primers complementary to the T3 and T7 promoter sequences which flank the cloning site, the inserts were amplified from 1-2 μ l of the colony lysate in polymerase
20 chain reactions for 30 cycles of 94, 55 and 72°C at 1, 2 and 3 min., respectively after an initial denaturation at 94°C for 4 min. The standard buffer, nucleotide and primer concentrations were 50 mM Tris-HCl (pH 8.3 at 25°C), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M dNTPs and 40 pmoles of each primer in 100 μ l reactions. PCR reactions were carried out on either a Perkin-Elmer Cetus (Perkin Elmer, Corp.
25 Norwalk, CT) or an MJR PTC-100 thermocycler (MJ Research, Watertown, MA). To carry out secondary screenings of the clones, aliquots of the amplification products were run on 1.5% agarose TBE gels (90 mM Tris, pH 8.3, 90 mM boric acid, 2 mM EDTA). Southern blot analysis was carried out on the gels after transfer to Gene-Screen Plus membranes (NEN, Boston, MA) using the alkaline transfer
30 protocol. The membranes were probed with (CA)₁₆ oligomers, 3' end-labeled with digoxigenin-dUTP using terminal transferase. A chemiluminescence detection system based on Lumi-Phos 530 as a substrate was used to detect positive hybridization signals following the recommendations included in a commercial kit, Genius (Boehringer Mannheim Corp., Indianapolis, IN). The membranes were
35 washed to a final stringency of 0.1 X SSC (1 X SSC = 15 mM sodium chloride, 1.5

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mM sodium citrate) at 65°C. The blots were then processed for immunological detection as described by the manufacturer. Once a clone was confirmed to be positive, the corresponding amplification product was then purified using QIAEX beads (Qiagen Corp., Chatsworth, CA) after electrophoresis on TAE gels (40 mM Tris acetate, pH 8.3, 2 mM EDTA) and directly sequenced using cycle sequencing (Delta Taq 2.0 Cycle Sequencing Kit, United States Biochemical Corp., Cleveland, OH). The sequencing reactions were carried out according to the manufacturer's instructions with the slight modification that T3 and T7 primers labeled at their 5' end with ³³P ATP (NEN, Boston, MA) using T4 polynucleotide kinase were used as sequencing primers. Sequencing products were analyzed by electrophoresis on 6% polyacrylamide gels containing 8M urea. The gels were dried and exposed to X-OMAT X-ray film (Eastman Kodak, Rochester, NY) for 1-2 days and developed. Primers flanking the repeat motif in each insert were selected to minimize hetero- and homodimerization; occasionally, the computer program Oligo (National Biosciences, Plymouth, MN) was used to help in the primer selection. The primers were synthesized by the Michigan State University Macromolecular Structure Facility.

Dog DNA Panel. To check the usefulness of microsatellite markers within and across different breeds of dogs, a dog DNA panel was established. The breeds to be included in the panel were chosen with consideration given to the diversity in origin and function of breeds that exist. Table I presents various characteristics of the breeds chosen for the dog panel (Alderton, D., *The Eyewitness Handbook of Dogs* (New York: Dorling Kindersley) (1993); American Kennel Club, *The Complete Dog Book* (17th ed. New York: Howell Book House) (1985); Clark, R.D., *Medical and Genetic Aspects of Purebred Dogs* (Forum Publications, Fairway, KS (1994), Walkowitz, et al., *Successfully Dog Breeding* (2nd ed., New York, Howel Book House) (1994); and Lee, M.P., *The Official Book of the Scottish Terrier* (Neptune City, T.F.H. Publications p. 158) (1994)). Five to ten individual dogs from each breed were selected for inclusion in the panel. Pedigrees were investigated to ensure that only dogs that had no common ancestors through four generations were included for independent representation of alleles. Ten, apparently unrelated, mixed bred dogs were also sampled. DNA was isolated from peripheral blood as previously described (Sambrook, J et al., *Molecular Cloning. A Laboratory Manual*. (2nd ed., Cold Springs Harbor, Cold Springs Harbor Laboratory Press) (1989)).

Table 1
Various Characteristics of Breeds in Dog DNA Panel

Breed	Country of Origin	Current Classification	Date of Origin	Height Range (cm)	Weight Range (kg)	Litter size
Cocker Spaniel	Great Britain	Sporting Dog	1800s	36-38	11-13	5
Labrador Retriever	Canada	Sporting Dog	1800s	51-57	25-34	7
Pointer	Great Britain	Sporting Dog	1600s	61-69	20-30	6-16
German Shepherd Dog	Germany	Herding Dog	1800s	57-62	34-43	8-10
Shetland Sheepdog	Great Britain	Herding Dog	1700s	35-37	6-7	4-6
Beagle	Great Britain	Hound Dog	1300s	33-41	8-14	5-6
Greyhound	Great Britain	Hound Dog	3000 BC	69-76	27-32	10-15
Scottish Terrier	Great Britain	Terrier	1800s	25-28	8.5-10.5	3-6
Doberman Pinscher	Germany	Working Dog	1800s	65-69	30-40	8
Siberian Husky	Siberia	Working Dog	1800s	59	16-27	3-7

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Analysis of Microsatellite Variability. Amplification of the correct target was verified by comparing the product obtained from genomic DNA to that obtained from the reference clone. The variability at each locus was tested by amplification of DNA from the dog panel. PCR conditions were 35 cycles of 94°C, optimal annealing temperature (50-60°C) and 72°C at 1, 1, and 2 min., respectively after an initial denaturation at 94°C for 4 min. in the standard PCR buffer conditions described above. 100 ng of genomic DNA was used as template in each reaction. 10 µl of the PCR products were analyzed by vertical electrophoresis using a modification of a SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) protocol (Laemmli, U.K., *Nature* 227:680-685 (1970)) as described previously (Tas, S., *Anal. Biochem.* 188:33-37 (1992)). An HSI SE600 vertical slab gel electrophoresis system (Hoeffer Scientific Instruments, San Francisco, CA) connected to a cooling unit was used. The gels were poured between 16 x 16 cm. plates using 1 mm spacers. 1.5% acrylamide stacking gels of 2-3 cm were used on top of 12.5% acrylamide separating gels with 30:0.8 acrylamide to bis-acrylamide ratio. The gels were run at 40 mA through the stacking gel and then at 70 mA through the separating gel until the bromophenol blue dye reached the end of the plates, for approximately 4 hours. The amplification products were visualized after silver staining with the Silver Staining Kit (Bio-Rad Laboratories, Richmond, CA). This procedure resolved differences greater than or equal to 4 bps in the size of amplification products in the 75-250 bp range.

Results

Screening 110 plates resulted in the isolation of 1064 independent clones that were confirmed to be positive on secondary screening. Using 600 bps as the average insert size and 500 as the average colony number per plate, it was calculated that 1064 positives reflected an estimated incidence of one CA repeat clone every 31 kilobases in the dog genome.

The first 14 CA repeat loci for which primers were designed are presented in Table 2 together with the optimal annealing temperatures.

Table 2

	Marker Locus	Primer Pair	Repeat Motif in Reference Clone	Product Size (bp)	Annealing Temperature °C
1	D00101	ACTCTTCTCCATCTCCCTCTGC TCGTTGGGTTAAAGCTCTGACC	(CA) 9	150	65
2	D00401	TGCCCTCACCAGGTGTATAGA GTGTGAATATGATGTGTCTGAAAA	(CA) 22	90	58
3	D01205	AGCATGATGCCCTTCAAGGTC GGATCTTTACCCGCATGTTCC	(GT) 16	201	58
4	D01902	CCTACTAAATACAGAAACG AACTGTTAGAACTTAGACATGC	(CA) 18	129	55
5	D02001	GTTCTCATAGAAAGGATGAGGC ATATTCTCTTAGGTTAGACAGCAGG	(CA) 20	270	67
6	D02005	TCTAAATATGATTATGTATGCGT CACTTTATAACAACATATTCAAAT	(CA) 13	119	55
7	D02011	GGTCACCAAGCTAAGAATGTTGC GATCTCTCTTGCTATTGCTC	(TA) 7 (CA) 13	238	55
8	D02012	CTGAGATGTGTCAAAAAGTCCTTTTCG TTGCCCTACAAAGATCCCTACATGCC	(CA) 15	171	60
9	D02202	TTAAGCAGAAGCTCCGCTGC AATTTTGGTGCCCACTATGGAAGCC	(CA) 12	91	60
10	D03709	ACATTTCTGAGTGGCATGGCT ACTCCCAAATCTTCACAAAGGAA	(CA) 9	86	58
11	D03805	GTCAACAGCTTAGAAGTCACCA ACTATTATGCTGTAGGGGTGCAA	(CA) 12	90	58
12	D03908	TACACCTGACACTTGTATCC GTGCTTGTAGTCCATGACC	(CA) 13	94	58
13	D04403	CTATTGATTTTTCCAAAGC GTCTTTCATGTTTTTCATATACTC	(CA) 15	130	50
14	D04702	GTCTTCCAAGTGGTAAGAGCCTACC ATCCTCCTCTACCCCTCAGAGCC	(CA) 12	112	60

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The complete set of microsatellite markers is set forth in Table 2A below. These markers were identified and the primers designed as described above.

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Table 2A

Marker Locus	Sns Sequence	Asn sequence	PCR Product (bps)	Motif
C00103	CTACTCTGTATTCCATCAAT	ATTTTCCCATTTCTCACTGGT	242	(GT)21
C00104	TGACATAAGCTGTGAGAAGAC	ATTGAAACTGATAGAGAAGAG	140	(GT)9
C00111	TACGGAOCCCACTACTGA	TCCAAGGGAAGTCATAGAAC	226	(GT)11
C00111	AGCTTCCAGGTCTGGTTTCCAAAG	TATCCCAAGCTTAGAGCCTGGCA	174	(GT)11
C00113	TTTGTGATGGCTGAATAATA	GAATGGATAAAGAAGATGTG	82	(GT)14
C00114	CTGCTTCTCCCTCTCCCTATGT	CTACCACAGCCAATGTTGATTGA	140	(GT)12
C00203	AGGGTGCCTAACTGACTGAGCC	TTTCAAAATGGGCTTTCCTTT	162	(AC)17
C00203	AGGGTGCCTAACTGACTGAGCC	TTTCAAAATGGGCTTTCCTTT	162	(AC)17
C00215	TGCCCCITAAAGATTTTATTT	CCTGCATCGAACCTGCTTCT	127	(CA)10ACT(AC)12 (AG)4
C00217	TCCTGCATGGAGCCTGCTTCT	TGTGTATTGATGTGCTACTTGGT	181	T11A2G(AT4)(AT 3)2(AT2)_(AC)10 _(GA)16
C00304	GCACCACTGTAAACCCTTGAAC	TCCCATAGGATGATGAATAATA	181	(CA)4TA(CA)12
C00403	ATGAGGCCTACTTCTCCCTC	GACTTGCTGTATTGGTTACACT	123	(TG)11
C00412	ATCAGTCCATTCTGATTGCTATC	GAAAATGGCAGTTGTACCTGAATCT	209	(TG)13(TA)4
C00501	ATCACATCCAAATCAAGACTAT	TGTCCTATGCCTGTCTATTAT	172	(AC)15
C00502	TGACTTTACCTTACTTCACCTT	AGGCAACTTGGTTACAGATTA	109	(CA)3T(AC)2C(C A)6
C00505	CAGAGCCTTCAGATAACAGTA	ATTATTCCTTCCCTTTTCTAC	230	(GT)9T(TG)4(TA)4 (TG)7
C00506	CATATCCATCCTCCTAAACTTTC	AGTGCCTAAAACAAAGAACTG	173	(GT)2A(GT)9
C00602	CCAGGAAGTTATGATTCTAAATGT	GAGCTTGCTTCTCCCTCTGCC	214	(AC)7(AG)8
C00603	CTTTTCTATTGTCAAAATG	ACAAGATGAATACAGTTG	107	(TG)12
C00607	AGTCCACATCGGCTCTCT	TGCTGGTTCTCTCTTGTGTCTTAT	169	(CA)9TA(CA)4
C00613	GTGGAGCCTGCTTCTCCCTCTG	CTTCCAAGTGCAACACATAGC	191	(GT)7(A3T)n
C00802	TACCTGAGTCAGTTTACCTAGCA	GTTTCTACAGTCAACCAGATG	185	(GT)19
C00803	TAAGAGTTATGCCACTTGACC	CCAGGGAAGAGACCAGTATATGA	100	(GT)12
C00901	TAAAGGTCCATTGATAGAGGA	TGATCCAGGAGTTCATTCTT	105	(AC)12
C00902	GAGCCTGCTTCTCCCTCTG	TGTTTCTTCAATGACCTTTCAAG	175	(CA)14
C01001	ATGGGCTCCAAGAAATAGCA	ACCAGAACTTCATTGTCTCC	219	(GA)12
C01003	GAAGTAAATCAACAAACAATCA	GAAGCAAAAGTATAAGAGCTGTG	87	(AC)11
C01201	ATTCTTCTATGGCTAGGCAGT	TGAGTTTCTCCCTCTTCTCT	150	(GT)6A(TG)5A(TG 3)
C01207	AGACCCTCTGCTCCCTCTT	TGCCCTGAAATGAACAATGA	84	(GT)15
C01212	AGGTGTTCTCACTCCTCATA	CTCCCTCTGCTGTGTCTCT	115	(CA)10
C01304	CTGAGCAAGACCCATACCACTT	CCTCCCAGAAACAATCTATTTC	180	(TG)7TA(TG)4
C01305	GCATGAGATAAGACACCCTGTT	TTCATTCTCCTGCTCCTGTG	136	(GT)9
C01403	GAGGCTGACAACCTGTTTGCTA	GGAGATAAATGATGAGAACTCA	284	(AT)2T(AT)7CA(G A)4_(CA)7(GA)2(CA)2
C01406	GATTTTATTCATTTATCCATGAC	CTCCCTCTGCCTATGTCTCTG	107	(CA)16(GA)16
C01406	TGGTGAAAGTAACTAAGAACA	TCCCTCTGCCATATGTCTCTG	150	(CA)16(GA)17
C01409	GTTCTTCCCAATGGTATTTA	TTGCATAAAGGCCAGCAAACT	246	(CA)6A2(CA)3
C01505	TCTGCCTATGTCTCTGCTGT	ATAAGATACACGAACCATTAGCC	109	(GT)13
C01601	CCTGCATGGAAGCCTGTTTCTC	CATTCTGGAAGACATACTGTA	145	(GT)7
C01606	ATGCTGTTGATTACACAGACC	ATCACTTCTGGTATTACAC	109	(GT)19
C01801	TCTGATTTTCAACCCTTGAAC	GCAGTTTCTGTCTCTCTT	144	(TG)10(GT)9
C01802	ATGCAAGTTCTAAAACCATACTG	TAGTGAAGACAGGATTGTGTTG	137	(TG)19
C01908	ATCAAGTCCACATCAAGACCT	AGTGTATGAGGGGCATAAGGAA	189	(GT)10
C02005	GAGTAAAGAAAGAGTTTGAACAAT	AGTTGGAGAAATGAGCACTTA	146	(GT)10
C02122	ATGTCAGGCTCCCTGCATGG	GTTAAATGTAAGATGTC CAGCCCTT	149	(CT)4GT(CT)6(GT 6)(CT)3
C02401	CCAGACCCAATGACATCTCC	ACCCAGGTGCCCTCTTATCC	236	(GT)18
C02509	TGGCTAAACACCTCTGACAT	TGGGATACAAAGTAAATGGAAC	189	(CA)18
C02511	GACATGATTACCACATTCATC	GTACAATCAAGAGACTGACC	97	(GT)16
C02601	CTCCCTCTGCCTGTGTCTCT	TGTTAGTCTTAGCCATTCTGA	144	(GT)8(CT)3_(CA) 12
C02604	CTCACCCAGAGGATGCTTTGAA	TTAACCTGAGAACATGGCACAA	190	(CA)17
C02608	AGGAGCAGGTTTGTGGTTG	TACTTCTGGTCCAACATTTCC	110	(GT)19
C02705	GAGTGATTCTCATTTCAAAAAGGGA	TCAAAGGCACTTTCTACTGTGTA	116	(GT)10
C02709	CTCTGCCTACGTCTCTGCC	CACCAATATGCTGATATAATTCT	142	(CA)18
C02711	TCTCATTTCAAAAAGGAGATGC	TTTCAAGGCACTTTCTACTG	109	(GT)10

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Table 2A (cont.)

C02712	GCTTGGATGCTATTGCTCAA	CAATGACTTGGGAACTACATTC	136	(GT)22
C02802	CCCTGCATAGAGCTGCTTCT	AGCTTTTGCTTATTATATGCTTG	186	(GT)6(CT)2CA(TG)8(TC)2(AA)T
C02803	GACAAGAACAGGTATGAGAGC	TGTTGAGTGTAAGATTCAAAGC	118	(CA)12
C02806	TCCCTCCTCCTGTGTCTCT	CTACACCTGTGAAACTACCA	139	(GT)11GAG(A3T4)(CT)A6(TA2)3(TA3)
C02903	CCTACATGGAACTGCTCTTC	TGTCTTTCCCTCAACAAGATG	167	(TC)4TG(TC)5
C02911	ATCATGGGAGAGGGTGGTAT	GGGTAGATAAAGACCTGTAAG	122	(CA)16
C03001	TTCAAGATTAAATGATGCTTAGG	GAGATTCTCTCCCTGTACCAC	153	(GT)7(GA)17
C03102	ACTTGTGTACCCCTTTTACC	CCTGCTTTTATGGAGTTTACA	108	(CA)5TA(CA)15
C03104	TCCTCTGCTGTGTCTCTAC	ATCAATGAAACAAAAGGAACAGTA	147	(GT)19
C03109	CCTGCATGGAGCTGCTTCTC	CACACCAATTAAACAATAGACATT	185	(GT)16
C03301	CCATTCCCATAGAGAGGAA	ACCTAGCCAGGACTGGAAAG	118	(CA)7TA(CA)11
C03302	TGAGTATTATGACCTGGAGGGT	TCAGTAGGTTGTGTCTAGCCT	97	(GT)11C(TG)5
C03302	TCCTAATGATACAAGAACTTCAC	TCCAGTACCCTCCAAAGATGT	185	(AT)11(TA)8(CA)16
C03304	ATTGGCATCATTCCTGGTCA	TGGAGGCAGCTTAAATCTCAACA	95	(AC)16
C03308	TGATAAGAGTGTGAACAGAGAAGA	CTAGGAGATTGTACAGGTGCT	275	(GA)-20
C03401	GGTCATCTTTATACCATCAATTAG	CTTAAATGCTGGCAGATGCTAT	104	(CA)10
C03404	CAATTCTCTCTATGCCTCTTTGT	TCTTCTTGATTACAGCCAACTCT	171	(CT)4T(CT)2GT(C)10(CA)18
C03501	TCGGAGATGGAACTTTTGTAAAGAG	TCTAGTGGAGTGTCTGAATTTG	106	(GT)21
C03507	ATCTCGTAATTTCCATAATACTTA	ATCAAGTCCACATCAGACTCC	161	(GA)2(CA)5TA(CA)8(GA)6
C03508	TACTCCAATGGCAACAGTTTA	CCTTAGACCATCTACCTCTTTTC	110	(CA)5G(CA)17
C03509	CATTCTGCTCATCTCCATAAG	GGCACAACCTAATCTATTCTAT	188	(CA)15
C03510	OCTGCATGGAGCCTGCTTCTC	TGGCTATTTATGGAGCATCTCTT	156	(GT)19
C03512	GAGCCTGCTTCTCCCTCTG	GAGACCATAATTACAAATCTTC	113	(TC)12ATGA2T(A3)T3...An
C03601	AGCCTGCTTCTCCCTCTGTC	TGTTGCTTACCCTTCTGTTAGA	151	(CT)3(GT)10(CT)2
C03607	AGTTCCATCCACATCGTTTCCA	AGAAAGAGCCTAGATGCCCAT	141	(GT)18
C03810	TGCTTCTCCCTCTGCTGT	GGCTGTAAGACGCAGATTCT	134	(AC)17
C03814	ACATTGGGTTCTGATGGAG	GGCAGTTTGGTGATGTCTATCAA	237	(TG)19
C03815	GTGCATGGAGCCTGCTTCT	AGCTTAGCACCTGCATGGA	161	(CT)6...TA3)2(TA5)2TA4)TA3)4
C03907	TAGTGCTCATGGAGCCTTTCA	TATGCTGATTCCACCTACCTC	83	(GT)13
C03909	TCAAATCAACTCGTGTTCGT	GGATCTGATAATCCACTTTAGA	71	(TG)8
C03913	GAAGGGACAGAGAAAGAAATGAC	TGTAAGGGCTGTTACCTCTAATC	333	(TC)13(AC)12
C04003	GGGTCTCCTTATCACACTG	AGCAACACTTGACATTATT	135	(CA)12
C04007	ACCAAATGAGCCACTTAGGT	CCTCTGCCCTTTCTCTATG	109	(CA)11
C04103	AATGCTGTGGAAGGTGAATGATA	ATGGAAGCCTGCTTCTCCCTCTG	224	(CA)8(GA)4
C04107	TCAGCAACTATACATTTAAGAGCA	CTGTCCCATCTAAAGGATAGG	160	((GT)8GA/GT)11
C04107B	ATCGAGTCCACATCCTTG	CATTTACTGTTTGTCAAGTAGG	120	(AG)11
C04107C	TGCGAGATGAAAAGTATCCTC	CCTGTGCCCTCAAGATAGATG	250	(CA)18
C04201	GAGTTCCCTTCTCCGCATCTAG	ACTATTGAGAAAGCAGTACAACCT	120	(GT)6A2(GT)14
C04208	ATCCTAGTTAGGCATGTGCTT	GGTAAATTACAGCAGGTGAT	205	(GA)2(AC)11
C04302	TGTTTATTACTGAGCAGACATC	GCTTTTGTTCCTTCAAATAC	168	(GT)21
C04601A	AGAACTATCCAGCTATTATAGTG	CTCTCAGATATGACCAACCTA	214	(TG)18
C04601B	ATATACTTTCACTCTCCATGCAA	AGAAGAGGAGTCTTTGGATG	139	(TG)18
C04704	CAGTTGCTAAGAGGTAGGTC	GTAATGATTACCATAATAAGGT	114	(CA)13
C04716	TTCTCCCTCTGCTATGTCT	AGCACCTGCTACTGTTCT	133	(CT)3(GT)9(AC)1ATC)A(TA3)2(TA8)T3A12)
C04802	TTACCAAGCTAAGCCTGGCA	TGGAACCATCACTGAAGGGA	150	(C6A)C6T)AC)20
C04802	AGACCACCGAATGGATGGAGT	TGGAGTAAGTAGCAATCCTCT	144	(AC)17
C04803	CTTTGGTCTCTGGTGCCAATAG	TGGACTTGTGATACACCOCAT	207	(CA)17
C04806	GCCTCACTCATCATTTTC	GAACAAGAGATTCAATTTGCTATCA	180	(TG)18
C04903	ACTGCAATAACCTGTAGAGTGCT	ACCAATCACTTCCCTCATTC	157	(AC)16
C04904	AAGACTTCACCACTCACAGTCA	CTGGCTCAGTGTGTATGAATG	143	((CA)6T(CA)11
C05101	CTCTTAACCGACCTTGACACC	AGAAGTTGCTTATGAAGTCATGT	208	(AC)15
C05102	AAGCTGTGATGTGGCTCTCAAC	CAATGGGCAGAAACAATGAGGA	171	(AC)20
C05103	ATTGGCATTATCTTCATTGT	AAGAGGAAAGAATCTGTGAACCT	196	(GT)16T(GT)2A(TG)5
C05110	TGGAGCCTGCTTTCCCTCT	ACCCTGAGACCATGAGCTAAG	185	(CT)3(GT)8

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Table 2A (cont.)

C05112	GTACTAACTCCTTGCAATTCATC	GCCACCAAGTGTITTCATGTAAT	138	(CA)2CG(CA)9
C05201	CTGCTTGAACACTGCCATC	GCCATGGAGCCTGCTTCTC	167	(CA)18
C05204	GAGCCTGCTTCTCCCTCT	TACCTGTCAACATACATAGT	164	(CT)2(GT)14
C05205	ATCACGACCCTGAACCTAAO	CCTGCTTCTCCCTCTGCC	224	(AT)310 (AT)31 (AC)10
C05206	TGACCTTGGGAAGCTGGAO	CCATCAGTGGTGTATCTOTA	151	(GA)2G(GT)14
C05302	OAGCCTGCTTCTCCCTCTG	CCAGGATTTGGAAGGTTCT	178	(GT)15
C05303	ATCAAAGTGACACATCATATT	TGAAAGGACGCTGAATTGG	132	(AC)18
C05305	TATTGCATCCTGCTTCCAGA	CAGCCACGTTGGCCCTTCT	105	(GT)14
C05306	ACAATAGCCTAGATATGGAAGCA	GCTGCAAAATAGCAAGATTTCAT	148	(TA)3(CA)13
C05307	TGAAGTAGTAGCCTAACTGACA	TAATCCTAATGCCACTCTAATGGT	300	(AC)15
C05401	CGGTGCATGGAOCCTOCTTC	CTGAACCATCCAGATGTCCAGA	152	(GT)13
C05403	GGTGCATGGAGCCTGCTTCT	CACCTACCTCCCCCTTCTGCAA	141	(CT)3(TG)10
C05404	CTGTATGGAGCCTGCTTCTC	CCTTGAAGGATATTGTGTCC	138	(CT)3(GT)13(CT)2
C05405	CTAAACCACTGAGCCACCTG	ATGTGTAAACAGAAGCCACTAA	263	(OA)2(CA)6TG(CA)7
C05406	CAGGGATCTTCTTTTAGCAT	ATTGATGTTTTGTGAGATTTC	280	(TG)3TA(TG)7
C05407	ATTATTACTGGTGGCTTATTAGA	TCATGGGTCTAAGTGTITTOGA	101	(CA)8
C05409	CGGTGCATGGAGCCTGCTTCT	GGGAGATAGACAATCACCAAAT	231	(CT)13(GT)7(CT)2
C05410	TTTCAGTCCAGCCAAATGAAC	CCTGGGATGGAGCCTGCTTCT	183	(CA)8
C05414	GAGTCCACATCAGGCTCC	GCTGTTTACACAAAACATAGAAG	150	(GT)11
C05415	GCCACCCAGGGATCTTAAAT	CCATTACCTACATGGTTACTT	73	(AC)7
C05503	TACCACTCTGCTTGGACAT	ACTAATFCCAATGTACTGTTAC	163	(AC)9
C05504	GTCCACTTCCAATTGCCGTT	AAGTACAGGAATTCTGTTATGAG	234	(CA)2G(AC)8
C05505	AATCTCTCAAATCTCCTCCAT	CTCTGATTCCTCTAGTTTCTTTCCT	243	(TG)11T3(GT)4
C05506	CACATGGGCCAATTCTATAA	GTATTGGTCAGGATTCTCCAG	136	(CT)17(AC)7C(CA)10
C05509	TGTCGGTAGCATAGCATAGAA	CCTCAGTTTTACATGAACTCA	78	(CA)14
C05601	CTGCTTAGAGTGTCTGTACCAC	CTCAGCTCCTGGACACTTCCT	168	(AC)19T(CA)4
C05602	TCTAGAGGATCACATGCAA	CTTCTGGACTCCTGCCCTCC	105	(TG)15
C05604	CAGATGTTTCAAGATGATTTAATAG	ACCTGATATGTGGCATGTTGT	227	(AT)4(GT)7
C05606	TATAGTAGGATCTTGTGGTTG	ATCGAGTCTCACATCGGCTC	194	(AC)23
C06105	AATAATGAAAACAGCCAACTT	ATCATAATGATTGAATGAAT	98	(GT)12
C06106	AATAATGAAAACAGCCAACTT	TTATTAAACCCACTGAGCTACC	151	(GT)12
C06114	CTCCCTCTGCTGTGTCTCTG	GGCTCTTCTTGTATCTTT	140	(GT)14
C06201	TCTCCTTCTGCTACTTCTCC	TAGTGGTGGGTTGAAAGAG	138	(AT)11
C06204	GGCTGCCCTCACACATATT	ATAACATCTGGATTGGGTCTA	105	(CA)10TA(CA)8
C06213	CTGATATAGGTAAATTGCATTTTG	CTGGAGCCTTTAAGGTCATT	177	(GT)14
C06216	ACTCTCTTCTGGCTTGTAGATG	TAGCACTCTCCCTTCCCTTA	167	(GT)15
C06404	ATCAACCACAGCTCCTTCTT	TTGGGGAGTAGCTTCATTTCCTG	128	(TG)18
C06405	GAAATGAAGTTATGAAGTTTG	AGGATTAGTGAAGTTGTTTACC	143	(CA)11
C06406	ACCAAATGTCAATCAATAGATGAA	CTAGACCCATCCATGTTGTTG	131	(CA)16
C06504	CCTGAATAGAGCCTGCTTCTCC	TGTTTATTGCCCATTTGAAAA	214	(CT)6(GT)7AT(GT)2(CT)2CATG(AAT)3
C06508	CCATGAATGTTGAGTGTCTCAT	GAGCATGCTTCTCCCTCTG	186	(CA)8(OA)13
C06511	ATAGTGAAATGCCCTAGTGGT	TATCATACTGCCCATTAATGTG	114	(CA)11
C06513	TGTTGCTCTCTCTGCCCTAAT	CTTCAATCTGTTGGTGTCTAT	161	(CT)9(CA)10
C06602	ATCCTTAGATGTAGACCCTTAG	TGTCATCCAGGCAATAGAAT	137	(GT)11
C06605	TCTCCTTAGGGACTGTACCC	GCATCACAGACGTGTCAAGAAC	131	(GT)19
C06610	CTCAGAATCAGCAGCAGGTGCC	GTTGCTAAGTTACAGACATCACCA	206	(CA)10
C06905	CAGAAACTGAGATGTGTCAAAAGTCC	ATGCCATGTTCTGATGCTCTTG	166	(GT)14
C07002	TTCTGGATGAACATACCTTTG	TGGTCAGGGGTAGAAGAGTG	81	(GT)12
C07003	GAGCCTGCTTCTGCCCTCC	GTATTAATGGATGGATTGCA	156	(GT)25
C07004	AGTTTGAACATCTTAAATTGAT	AATGCAGAATCCAAGAAATAGAG	118	(GT)12
C07010	CTAGTTCATCCACATCATTG	ACAATCCAAGTGTCCATCAAC	138	(CA)15
C07011	TTCTCCTCTGCCCTGTGTCT	GTATCTTTTATACCTTGGACCTAT	215	(CT)6(GT)15(AAT)3
C07013	GAAGGAAGCCACCAGTAAAGT	TTCTTAGAAAGACCCGAGTA	138	(GT)11
C07102	AGTCACAGAGGCGAGTGTGG	ACATCCGCTTTAATTGTTC	118	(GT)17
C07104	GTAATCTCCATTCAACACAAGTGA	CGGATATAAAGGTGGGTATT	187	(CA)9
C07108	TGCATACAGTATCAATTTGTGA	GGATAGAGTCCACATCGG	168	(GT)10GA)9
C07212	ACTATATTGACAAGTATGCACAAGA	GAGCCTGCCCTTCTCCTCTG	183	(CA)20
C07301	GATAGATGAATGGATAAAGAAA	TTAGCATAACACTCTCAAGTT	135	(GT)11
C07302	ATCACTAAACCACCACAGAG	AGGTAAGGCGAAAAGAACTT	129	(GT)9

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Table 2A (cont.)

C07304	CAGTTACATATACCATTAGCCA	TGCTCCTCTTTGTCTCCA	109	(CA)7TACG(CA)10
C07308	ACATTGGGCTAATTTAATAGAT	GTCCTGGAGAGCTTATAGTAGACA	127	(CA)11TA(CA)3
C07403	TGCCATCTTCTGATGCTCTTG	TCGTGGTTCTTCTGGAATCTG	134	(CA)14(T3A)10
C07407	TCATTCAATCAAGTCTCAGTTAT	CTTATGGCTGGAGGTGTGTA	121	(CA)15
C07413	TTACACGACGGAAGAACTGTTATG	ACCCCATCAATCAAGAGAACTTA	120	(GT)18
C07415	AACTGTGTACTTCTGTTTCAT	ATTTAATCGACTGAATGTTCTC	101	(GT)8
C07502	CATCACCTCAGACTGTTAGTGT	GCATCTTCTTCTGGTGGGAAGA	180	(GT)11
C07902	GACTGATGGTGAAGGTGAG	TGTGACCAGTCTGTAACACTAC	91	(GT)14
C08103	CTTGGAAATGTAATGTGTGTA	CAGTTGTGATATTTTGTTCAG	91	(CA)12
C08202	ATGTTCTTAGCCAGTCATAAATC	TTTGAAGTTGGGATGTTCTCTA	203	(GT)13
C08204	TCATCTACTTCTGTGTAGCC	GGACATAAGAGGATGTGAGAA	113	(CA)21
C08411	AAGCAGATGCTCAACCACTGT	GAGGATCGAGTCCCAGGTCAG	174	(CA)13
C08413	ACTTAACTAGAGAGCGTGTGACT	ACCTACTTGGGTGTTTAAGG	135	(GT)13
C08601	ATATACTTTCACTCTCCATGCAA	AGAAGAGGAGTCTTTGGATG	139	(GT)18
C08608	CACAGAATACTGGAACTCATTAG	AGAATCTTATGGTTCGGTTTGG	155	(GT)18
C08903	AACTGACATCAACAGTCTGATAC	CGACTCTAAGATCGAGACCTC	186	(CA)16
C09004	CTACATGGAGCCTGCTTCTC	TGAAGAGGAATGGAATGACTC	138	(GT)11
C09107	CCTGCATGGAGCCTGCTTCTC	ACAAATAGGTGGTCACTTACTGAA	150	(CT)14(GT)7
C09109	TGGAGCAAGCACTTTCTATAAAC	GAGCCTGCTTCTCCCTCTG	148	(GT)16.....(GA)8
C09203	CCTCAAAATAGGAAGTGGCT	CAATCCAGTATGAAATGTTTAC	123	(GT)14
C09210	GGTGGCTCAGTGGTTAGCA	GGTGGTTATGATTGTACTTCTG	149	(CA)18
C09211	TCACCTACTGAGATACTTCCAT	CTGCCTATGTGTCTGCCTTC	204	(CA)7
C09213	TTTCACTCTGATTATATCTAGG	TGCATGGAAGCCTGCTTCTC	140	(AC)18
C09215	CCAGGAATAGACAATGCCCA	AACCTAAGACCTTTGTAATC	255	(CA)12
C09217	CTCTGCATAATGCCCTCT	AAGACTATTTATTTATTCATAGAC	80	(TO)11
C09220	CCTACTGTTTTCTGTATTGGCA	CTGCATAAAGCCTGCTTCTCC	165	(CA)4TA(CA)8
C09303	TCTGTCAATGGATAAAGTGGAT	TCCAGTTTATTTCAAGTAGTTAC	129	(CA)13
C09304	CTAGATTCATCCACGTCACGT	CCATCAACTGATAGGGAAGAT	129	(GT)12
C09305	TTGCCATCACTGATACAAGT	TTATTTCTCTTGCATAAATAGCT	181	(CA)9
C09307	TTACCTTGGCTATCTATCTAT	CTGTTCCATCTTTTCCACCTTA	164	(GT)5G(GT)12
C09309	TGGAGCCAGTTTCTCCCTCTG	TGTTTCTTGATTGGGTGGTA	141	(GT)13
C09310	TAGAGGATCAGGTCCCACGTC	GCAGTGGCAGGAATGAGTCA	264	(CT)11.....(GT)17
C09312	AACCTGGAAAAATGGATAATCAG	TGGAAAGATATTCACATTTCAT	144	(CA)9
C09314	GTCACTAAATTCACGTTATTGA	CTTTTCTCAGTGTGTCTCAGAA	228	(CA)8G(CA)6
C09403	AGATTGGAACCAAGAAATAGGAA	CTTGAGACTCTCTCTCTCTGTCC	182	(CA)9
C09407	TGTTAATCTTCTAATCTTCCAG	TCCACTGTTATTGGCATCACAT	104	(CA)16
C09413	TGGAGCCTGCTTCTCCCTCTG	GATCCACATCCCTGAGCTGA	202	(GT)9
C09601	TGGAGCCTGCTTCTCCCTCT	TGCTTCAAAGGACACATCAAGGT	138	(GT)17
C09607	GCTGGTTCTTTCTCTATTTATAC	TTCAAAGCTAGTCACTATTAGCA	131	(CA)13
C09609	ACTGCTGTTCTTTCTCTATTT	GGTAAATACTTGAGGAATTAACATT	102	(CA)12
C09610	CTAGCTTCTCCACTGAGTTCC	CAGATGCCCTCCCTAAAGATGTG	163	(GT)9
C09703	GCTTCAOGAATCTAGGGAACA	TGTATTCTCTATGCAATATACC	152	(CA)16
C09805	GTCCCTGCTTCTCCCTGTCTC	CACAGCAAGTGAGAGTGAGCA	156	(GT)10
C09806	GTAGTCTGCTTCTCCCTCTCC	TTCTCATATGTGGTAACTGAGTA	208	(CA)16
C09807	GCCAAATTAACCTATATTTAGAAC	AAGGCCTCAGACATGAACATAAT	176	(GT)6AT(GT)3
C09903	TCACATCCTCTTATCTGTTG	AACTCAGTGGGACCTTCAATA	148	(GT)5AT(GT)11
C09912	AAGATGATAGCTTGGTCAAAGAG	GAACCAGGTAATTTCTTCTATTGAA	135	(CA)8AA(CA)10
C10103	GTGGGGTCCCTACTCAGTG	GAGTGTGGAGACTGCTTAATA	289	(CA)11
C10104	GGCAGATTCTCAATACAGATTA	TGCTCTCATAATAGACGAATCACC	119	(CA)12
D00101	ACTCTTCTCCATCTCCCTCTGC	TCGTTGGGGTTAAAGCTCTGACC	150	(CA)9
D00103	GTACTTCTCAGCTTTCCAATG	CTCCCTCTGCCCTTGTCTCTG	177	(AT)34.....(GA)4(CA)12
D00109	TGTATGCTCAAGGATTATCTGG	TCTCTGTGCTGTGTCTCTGGC	127	(CA)17
D00401	TGCCCTCACCAGGTGTATAGA	GTGTGAATATGATGTCTAGAAA	90	(CA)22
D00701	CCTGCATGGAGCCTTCTTTTC	TGTATGCTCATTAAACCATAGTCTT	150	(GT)17
D00704	ATGGGGGAAAGCTGAAGGAGATCC	TGTCAGACTGATAAATATGC	459	(CA)23
D01004	TCCCTGCATGGAGCCTGCTT	GAACCAAGATTCCAGTTGCTA	246	(TC)12+(GT)12
D01204	TATCTACCTCTACACTCCTCTG	TGAGAGTTAAGGGGTTAATGG	589	(GT)20
D01205	AGCATGATGCCCTTCAAGGTC	GGATCTTTACCCGCTGTTCC	201	(GT)2A2(GT)16
D01208	ACTCTGACAAGGTTCTGGCG	GAGTTTATTTGGTGGTGTG	130	(CA)12
D01210	GCCACAACCTACACAATACTAA	TTCTACAGTGATGAATCCGAGT	213	(CA)10
D01211	GCTTTTGTTCCTTTAGTGA	GTTTCATAGCAGCAATGTCCAC	127	(CA)23
D01212	CATAATAATTCCCACTACT	GGAGCCTGCTTCTCTCTCTG	133	(CA)17
D01214	ATCATGTGAAAGCAACCTCTC	TTCTCCCTCTCCCTCTGCTT	254	(CA)5(GA)6

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Table 2A (cont.)

D01215	CCTGCATGGAGCCTGCTTCTC	ACGAGAGACTCCTAACTCTGAAA	260	(TG)17
D01504	CTGCTTGTAGTCTAAAGTAAGTC	CTGACTGAGCAGTGAATCTA	237	(TG)3(CA)(TG)(T A)(TG)9
D01505	CCAAAGGGTATGTTGTCTATTACT	CAOCATGAAGGATCTCTGACTA	157	(GC)9(AC)13
D01702	CTCCCTCTCCCTATGTCCTGTC	TCGAACCAAGAAATACAGTTCC	450	(CT)16(OT)19
D01707	CTGATACTCAAGTTCACTGCGCC	CTGGTACAGAGGGCTCAGATCC	396	(AC)10AG(AC)5
D01708	GTAGAAAGCACTGAAGACATG	ATTTGCTCAGAAATAGAGCC	279	(GT)12
D01715	TTACTGAAAGTGAATCTACCTGTC	TAACCTTCTCTTGGATGTGAAGG	192	(GC)9(AC)3AT(AC J7
D01901	TTGGGTGATAATATCTATTGCT	CCTGCTTCTCCCTCTGCTGCT	190	(CA)13
D1902	CCTACTAAAATACAGAAAACG	AACTGTTAGAACTTAGACATGC	129	(GT)18
D02001	GTTCCTCATAGAAAGAAATAGGAGC	ATATTCTCTTAGGTTAGACAAGAG	271	(AC)20
D02004	CTTCTCATCATCATTTTAC	GTAGATATTGAAGAAATGAACA	184	(CA)17
D02005	TCTAAATATGTATATGTATGCT	CACCTTTATAACAACATATTCAAAT	119	(CA)13
D02009	TAAAGTTTCTCATTTTCAAGT	ATCCTTCTCTTTTGGCTAATA	143	(GT)15(GA)15
D02012	CTGAGATGTGTCAAAAGTCTTTCC	TTCCCTACAAGATCCCTACATGCC	171	(GT)15
D02202	TTAAGCAGAAAGCTCCGCTGC	AATTTTGGTCCCACTATGGAAGCC	91	(CA)12
D02209	GCTCACACATGATCTTTGTATTCC	TTCTCCTCTGCTGTATCTCTGCC	180	(AC)10
D02210	GGGTCTGAATTTTGTTCAC	ACATCAGGCTCCCTTCATGG	160	(AC)11(AT)2(AC)5 (AG)3
D02211	CCAAGCATTACCTGATACCA	GAATAAATCCTCTGATTGTG	201	(CA)18
D02212	AGCCTGCTTCTCCCTCTG	CCTTAGTATCCAGTATCAC	213	(GT)12
D02214	AAGATTCTGTGAGACAGGATCAGCG	ACTGGAAGGAAAGATAGCCAATGCC	191	(TG)16
D02919	GGTGCAGTTACTTAAAGACAG	ATGTTTGAACACATAAGTAGG	123	A15T2A10
D03202	CTGTCAAGGTCACTGAGATTTAGA	CCAAGACTATAACCTCCACAT	156	(GT)15GT(GT)3
D03209	ACTGGAAGTGAAGGTTCAAGGA	CTGCACTGGAGGCTGCTTCT	300	(CA)3G(GT)21
D03301	CCACCACACTCCAGGTTCCA	CACGTAAAGTAGTTGAACCTTAC	231	(CA)17
D03505	GGCTCTCTCTTGCCAGAGA	CTGGACTTTGCATTCACTTTTCAG	133	(TC)4(AC)2(TC)3
D03601	GGAATCTGCTTCTCCCTCT	ACATGTGAGATGCTCAATC	185	(GT)20A(TG)10
D03707	AGAGCTAGATGCCCATCAA	TTCACTTAGGTAATATCTCT	156	(GT)19
D03708	TTGAAAGAGATAAGGAGTCTGAG	TGAGGTCCGACTTAGAGGAT	82	(GT)3A(GT)5
D03709	ACATTTCTGAGTGGCATGGCT	ACTCCAAATCTTCACAAAGGAA	86	(GT)9
D03805	GTCAACAGCTTAGAAGTCACCA	ACTATTATGCTGTATGGGTGCAA	90	(AC)12AAT(AC)5 A(AC)2
D03815	CTAAGATCAAATCCCACGTC	GATTGATCTGAGTTAGCAC	172	(TG)5(TG)8
D03821	CCACCCAGGCATCCCAAGA	ATCTCAGAGAGTTGGAATCAATC	190	(AC)19
D03823	ATCTGGCTCCCTGCATGAAG	ACTTGTTTTCCCTCATATCTGTT	151	(CT)10(TG)5(T A3)(TA4)(TA3)9
D03908	TACACCTGACACTTGTATCC	GTGCTTGTAGTCCATGACC	94	(AC)13
D04101	CTGCATGGAGCCTGCTTCTC	GAATATGATGTACCAGGTGTGG	171	(TG)16
D04402	CCAGGCACCCCTTTTCTC	ATCAAGTCCCATGTCAGGCT	179	(CA)18
D04403	CTATTGATTTTCCAAAGC	GTCTTTTATGTTTTCATATACTC	130	((GT)15
D04501	ACTAGAAGACACCAAAATGA	AGGAATCTGCTTGGATCTCT	176	(AG)4-(GT)3
D04503	GAACCTGTTTCTCCCTCTGCT	GTCTCTCCCTTTCCCTCTGATG	158	(TG)17
D04504	GCAATCTATTAGTGGGTCAT	CTGACTCAGAGCTGAAATGTAT	224	(TG)14(GA)3GC(G A)6
D04513	TTGTCAATTGAGGAGTGCAT	CCACTCCAGAAATGTATCTAAAC	96	(CA)3TA(CA)5
D04517	TTGACTAAGGGACTCTCAG	TGGGTGCTCAGCAGTTTA	254	(GA)3(CA)10(GA) 14
D04606	CTGCTTCTGTCTCTCCCTAAT	TCCTCTGCTCTGTTCTCTG	280	(CT)10-(CA)13
D04609	AGCTATCTCTTCAATTTGATCTATCC	CTAGAAGGACAAAGTGTGCTACTGC	225	(TG)10AG(TG)5
D04610	ATCCAAAGACAATTCAAAAG	TTGGGTCTATTCTGGGTCT	133	(GT)10
D04613	ATCTCACTCAGAGCCAAAGCT	CGAGTTCCAAATCTTACAGG	293	(GT)10(AT)7(AC)6
D04614	ATCAAGTCCACATGCGGCT	GTGGTCTTATGCTTTCTCTTATC	154	(CT)12(GT)12
D04616	TCTCATTTCTGTTTATGCTGT	ATOCACCCCTATGTTTATTGCAO	167	(GT)17
D04617	AGGATGAGTAGGAGTCAGAA	GCTATGCTTTGGGATGACGTG	271	(GT)14
D04702	GTCTTCCAAAGTGTAAAGGCTTACC	ATCCTCTCTACCCCTCAGAGCC	112	((CA)12
D04710	TCCTGATGGAAGCTTCTT	CATTCACTTCACTTGAATGTC	526	(GT)17
D04810	CTCCTCTCCCTCTGCTCTG	ATGAACCTTCACTTGGGCT	231	(TG)14
D04811	TCAAGTCCACATCAGGCTTC	ACGTGGTGGTATCAAGTCTCT	189	(CA)19
D04812	TCCTGATGGAAGCTTCTTC	ACTGGTTTAAATGGAAGCTTCA	190	(TG)11-(AT)12
D04813	TGAGTCAAGTAAAGCAAGCTA	TGAGTCAAGTGTGCTATCTGTT	122	(TG)10TAGTCTG TCTA(TG)7
D04907	TGATTGAGCTCCCAATAACT	CCATCAACCGAGTCTGTAAT	216	(CA)13
D04911	TGATAGACACTTGGGTGCTTCCA	ACTCTTGGCATTACTCCAAGGA	164	(AT)5(GT)9(AT)5C (AT)7

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Table 2A (cont.)

D05005	ACATCGGGCTCCCTGCAT	ACCGTCATGTGCCACA	232	(AC)13
D05008	TCCCTATATGGAGCCTCTTCT	GAAGCTCCTATTTGCCCTTCACCA	200	(CA)13
D05012	GAAACTTCATAGGCAGACAAATG	AAAGTACCTATGGTTGGAGCATA	136	(CA)17
D05101	AGGCATCAAGAAATATTGTGGGA	AGAAAACACACCCAGAGACAGG	165	(GT)16(GA)21
D05120	ACTCTCCTGTATAGACATCTTGT	AGCAGAGGACTATGGGAAATAAC	108	(TG)12
DX-4	ACATCAGGCTCCTACATGG	CTCACTCAGGTTACTTGGCTGC	170	(CT)8(GT)7
E00402	TCACCGTTTTACCAATATTCC	TCATTCCTGATCGAGTTCTG	212	(ATTT)5(AC)3(AG)11
E00409	TGCTTTTGGATGAGCTGAAG	TGAGAGGATCAGTTTCTGTTG	211	(CT)8(GT)8
E03906	AGCAGTGAAGTTTAATGAAATAC	GCTCAGGAATTACCAAGGAG	85	(CT)12
E03909	AGCACTTACAGGCTGTGGTCGT	GACTTCCAGTTGACTAAATAAGCTA	214	(CT)9
E03912	TGTGGAGTCAAGCTTCAGATTG	GCTAAACCACTGCACCACTGG	150	(TC)18
E03913	AAACAAGTGGGGAAGGGAGG	CTTGAATCGAGCCCTGCATTGG	117	(AG)4(GT)7
E03914	TCAGTCCCACATGCAGCTTCTG	GTOAGACCAAAATTGTTATTGTAA	202	(CT)16(GT)8
E03917	AGGGAGAACAGATACTGACTCAA	TAATCAGCCTCTAAGGATTCTGG	216	(AG)14
E03920	CTGTGTGAAGCCTGCTTCTC	AGCCAGTCAATGTGCCCTTA	132	(CT)9G(TC)3
E03922	CACATTTTACATAAAAATAATATGCCA	CAGTGCATGGAGCCTGCTTCTC	192	(AG)17
E03923	CTGCATGGAGCCTGCTTCTTCC	GTTTCAGCATCTGCACCAAGAT	172	(CT)14G(TC)3
E04001	TCAGCATGGAATCTACTTGAAG	GAATGTOAGTACAAAGGTAGG	76	(CT)11
E04007	GCTCATTGTGATTCCTTAAACAG	CTGGGTCGGGATGGAAT	202	(GA)5A(AG)15
E04008	GATAGCCTGCTTCTCCCTCTG	ACCAGTGATTCCTTCACCTG	143	(CT)12(GT)3
E04019	GGCCTCACTGGACATCTTTATT	TGGAGCCTGCTTCTCCCTCTG	116	(GA)13
E04021	CAGTTTGGAGTCTGCTTCTCCCT	ATCACTGAATTGCAGTTGTCA	182	(CT)10
E04104	ACTAGGCATCTCACATACATTATT	CCTGCTTCTCCCTCTGCCTAT	109	(AG)12
E04105	CCTGGAATGGAGCACCATGTC	ATACTTATGTCCCTGGCTCTG	168	(CT)8C2T2(CT)6
E04107	CTCCCTCTGCCTATGTCTCTG	CCAAGCAGTTTACCACGATA	110	(CT)12
E04108	CTTCTCCCTCTGCCACTTC	TCTTTTATTGACAGGAAAA	98	(CT)10.....(CT)6
E04401	CCTGGCATGGAAGCCTGCTT	GTTTTAGGCTACACTTCTGAGT	122	(CT)9(GT)3
E04402	TGAATCATTATGGTCTATCGTTC	TAAATGCAAGTCTTACCAGAGGAA	111	(TC)13
E04403	TGCATGGAGCCTGCTTCTC	CCTTTCATTGAATATCTGTCAT	123	(CT)11
E04404	GCCACATAGACACTTGGTGT	CGGATGGAGCCTGCTTCTC	114	(GA)12
E04407	GGAGCCTGCTTCTTCCCTCTG	CACTAGTAGCTTTATAATTGTCT	124	(CT)14G(TC)4
E04408	TGCTTCTGGAAGCTGCACAT	TGCATGGAGCCTGCTTCTC	144	(AG)12
E04409	AGCCTGCTTCTCCCTCTC	GTTTTAGTCTACACTTCTGAGTAA	111	(CT)9(TG)3
E04411	GAGATCGAATCCACATCAG	CCTACTCTCCACCATTTTGGC	166	(CT)11
G00203	CTCTGCTATGTCTCTGCT	TGTATGTCTATTTTGTGCCAGTA	164	(CT)13
G00402	GTTTGAACCCCTGCCATAGGTA	CGGAATCGAGTCCACGTC	175	(CA)5(GA)20
G00410	TGGAGCCTGCTTCTCCCTCTG	GCCAACCTTTACATCTGTGCTA	148	(CT)11
G00501	ATGCCACGTCAGGTTCTCTG	GTTGTTCCAGTATTCATTCTC	171	(CT)11
G00504	CCTGCTCAGCAGAGAGTCTG	GATTGGATTATTTGTCTTGG	161	(CT)14
G00508	AGTGCGTGGAGCCTGCTTCT	GATGTACTGGCCCATCTTCT	196	(CT)14
G00512	CAGGCTCAATGAGTGATGTTA	TCAAGTCTTGCATCGCACACC	158	(CA)15
G00602	CGAGCTGCTCAACCGCTCAAC	TGGAGCCTGCTTCTCCCTCTG	187	(GA)19
G00603	TTCTCCCTTTGCTGTGTCT	GTCTATGAGAGCACCAGTTCA	190	(CT)11
G00703	CTTCTCCCTCTGCTGTGTCT	AAGTTGTGTATTGATTTCATTCTG	206	(TC)6T3(TC)7CA/(CT)3G(TC)3
G00704	GGTCTCTGAATCCTGTCTAT	GTGGAAGCCTGCTTCTCTTTG	225	(CT)9T(TC)3...A17
G00707	CTTCTCCCTCTGCCTATGTCTCTG	GAAGGCTTAGCAAGAGTTGAAGA	189	(CT)13GACTATC A(TA)2(TA)5(TA)2 A10(TA)62
G00708	CCTCTCCCTCTGCTGTGTCT	ACCTCTGAATCAGGAAATGTAAT	132	(TC)12
G00709	ATCGAGTCCGACATAGGGTCACT	AAACAGTGTAAACAACATGCTACC	152	(CT)12(GT)4(CT)3
G00712	ATCGAGTCCCATGTTGGGCTCC	TGAGCAGGGGCAATAGGAGACTTC	226	(CT)9G(TC)3ATG(A2T)2(A3T)2(A4T)(CT)2A8
G00713	CTGGATGGAGCCTGCTTCTC	GCGTATCTAGTGATGCCACTTCT	194	(CT)10T(TC)3ATG(A2T)(A3T)2(A4T)(CT)4A4
G00801	TGCTTATGGTACTCTCTCTCAA	TCCCTGCATGGAGCCTGCTTC	184	(CT)12GAG(TC)3 ATG(A2T)(A3T)2(A4T)(CT)3A9
G00810	CTCCCTCTGCCTACGTCTCTG	AGAAGTTACTGTGTCCAAGTACAA	152	(CT)17(GT)4ACTA TCAT(A3T)2(A4T)3(A11T)

Table 2A (cont.)

G00812	CTGCTTCTCCCTCTGCCTGTATC	AGGAAGTGGCATTCTACATTAGCA	198	(CT)11(GT)3CTCA TC(A2T)(A3T)2(A 4T)(T3A6)
G00903	TGCTTCTCCCTCCTTCTGTGT	ATTGTGAAAATCCCTCCTTAGAAAT	142	(CT)11
G00908	CCTGGTGATGGAGCCTGCTT	GATGCTGCAATGAACACGAGAGCT	143	(CT)12
G01006	GAGCCTGCTTCTCCCTCTG	TTTATTCTCCCTGTGTCTT	113	(TC)18TATCA(A3 T)2(A5T2)A10
G01109	TCCTTCTGCCCTCACCC	AGCCCAAGTTATAGACAATGAT	112	(CT)18C2T2(A5G) 2A9GA4
G01204	CATAGGGCTCCCTGCATGG	AGCCATTTGTATGTCTTCTTTGTA	226	(TC)17(TG)3TCTC 3ATG(A2T)(A3T)2 (A4T)
G01303	CTGCTTCTCCCTCTGCTTTGT	GTGCTAGATGGGGCTTCCTC	118	(TC)17GTG(A2T)(A3T)(A4T)CT3A8
G01305	TAGCTGAATGAAAGGGCTGATAG	TGCTTCTCCCTCTGCCTGTGTC	181	(TC)16(TA3)2(T2A6)(T2A2)(T2A1 0)
G01406	ATCAAGTCCCACGTCAGGCTTCC	ATTCGAGTGTTTCTTCGAGAAAGTT	161	(CT)15(A2T)(A 3T)3
G01506	TGGAGAACCAAAATGAGTCTT	GAAATCCACATTATAGAGGTTAAAC	155	(TC)16(GT)2
G01509	CTAATGTAAACATTGTGTGACAACATA	CATGGAGCCTGCTACTCCCTCT	110	(GA)9 G2 (CA)(GA)2
G01511	CCTTGCTCACCATATCACACA	TTCTTCTCTGCCTCTGTCTT	153	(GA)3 GA3 (GA)3
G01515	TGCTTCTCCCTCTGCCTATGTCTT	GTGCAGGGCTCAATGAGTGATGTT	133	(CT)16
G01617	TGGGATGGAGCCACAAGTCA	CTTACGACTGTTTCTCAACCTG	240	(CT)10
G01621	CCACTCCCATCTCTGCTCAT	CCAACGACTGAACCTGTCTAT	134	(CT)4CA(CT)6
G01705	TGGAGCCTGCTTCTCCCTCTG	GGGTTGCCTCTTCTCCCTTT	125	(CT)9
G01707	TCATTGCCAGACCAAGGTGTC	GTGCATGGAGCCCGCTTCTC	139	(GA)9
G01709	AGGGAAGACCCGTGACCAT	GCTTCTCCCTCTGCTGTGTC	238	(GA)10
G01713	ACTAGAACTACAGATCAATCC	GAGAACAAATGGCAGTTGTCT	187	(CT)8
G01715	ATGGAGCCTGCTTCTCCCT	GGGTTGCCTCTTCTCCCT	128	(CT)9
G01717	TGGAGCCTGCTTCTCCCTCT	CTGCATTTCCCTGATGACAT	172	(CT)11
G01804	CCAAGGATCAAGAACCAGTTC	GATGCACTCTCCAGTTGAACATA	168	(CT)14
G01807	AGGATCGAGTCCCACATTGG	TCAGTTAGAGCATGAATCTTGTGTC	205	(CT)2GC(CT)12TT (CT)4GT4
G01811	TATGAGTTGGGCTCCTGGTC	CTGGGACAGTAACACACATTAGT	197	(CT)16TT(CT)3
G01817	AGTCTGTGTCAAGCTCCAG	ATAGTGCACTCTTTTCAAGGAC	152	(TA)6
G01901	CTCCCTGCATGGAGCCTACTT	CTAGAGTTCTCTCAAATCTGTCA	130	(TC)11(GT)2 (TC)2
G01903	AATTAGCAGGAGTCTGTTTC	GGTACTTGGGTTTTAGAATAT	165	(CT)4T2(CT)3
G01905	TGAACCTGCTTCTCCACTG	ACGACTTGAGCCACCCAGGTA	169	(CT)9 (GT)2 (CT)2
G01906	GAGTCTGCTTCTGCCTCTG	CTGTACACTCTAAATGGGTCATT	152	(CT)9(A3T)2CT2A 6
G01918	TGTCTCATTCTAGCTGCTACATT	CTTCTCCCTCTGCCTGTGTC	106	(GA)18
G01920	TGGAACATATCTTTTGGGTGACC	CCTGCTTCTCTCTCTGCTGTG	233	(CT)23(CA)6(G A)7
G02002	AGGATCATTGGCTAGACAAAC	TACATAGTTGGGATCGAGTCC	248	(GA)10
G02007	TCCCTGCATAGGGOCTGCTT	GAATAAAACCTAGACTGGCTGAAG	128	(CT)2GC(CT)7
G02106	CATGGAGCCTGCTTCTCCCTCT	AAGGCAGATGCTCAACCACTGA	159	(CT)9
G02107	CTGCCAGAGAGAGTCTCCAT	TCGAATCCCATGTCCGGCTC	189	(GA)10
G02108	CATGGAOCCTGCTTCTCCCTCTG	AGAAATATCTTGGCTGCAATGCTT	146	(CT)13
G02111	ATTGGAACATATGCAGCTAT	CCTGTGTCTCTACCTCTCTGT	163	(CT)13
G02202	GGATCGAGTCTGCTGATCGAG	CTGAOCCAAAGGCACTCAACAG	177	A15(GA)9
G02204	ATCAGGCTCATCCGCATCAG	ACATAAGGAACTTCTCCATCCAT	200	(CT)9
G02501	GAGCCTGCTTCTGCTTCTGCC	GCCTATGGTCTTATGGGTGTTC	132	(CT)9
G02504	TAGAGGATCGGGTCCGOCCTC	TTACATGGTCTTCTTTTGGGT	197	(CT)16
G02506	GCAAGAAACATACACTCAATAGG	CCCTCTGCTGTGTCTCTACC	179	(GA)14
G02509	GAGGATCAAGTCCCATATTO	GTAGGCAOCCGTACAATGAT	135	(TC)9
G02512	CGCTCATGCAAGTCATCACAT	ACACTCTGGTCAAGGCACTC	125	(CT)15
G02513	CATTTCTCAGCATGTATTATAGAT	GTCCGGCTCCCTGCATAGG	120	(GA)14
G02602	TACTCTGATGCACTCATAAGG	TGCTTAAACCTACTCTCAG	123	(CT)14
G02610	CTTTGCCAGTTATGGGTCTGTG	TGCTGTGTCTATGTCTGCA	132	(GA)16
G02616	GCCTACTTCTCCCTCTGCCTATG	CTGCTTCTCCCTCTGCTTTTC	163	(CA)2(GA)10
G02619	CCTGCTTCTGCTTCTGCTGT	TTAGTTTTCACCAACTGTAAGG	154	(CT)9
G02620	CTGCATGGAGCCTGCTTCTCT	GAATTGTAAAGTTTCAACTGCC	144	(CT)9(CT)4
G02702	ATCACAACTAACAAAAGGCT	CTCTCCCTCTGTCTGCCACTCC	142	(GA)12

Table 2A (cont.)

G02704	ACCCAGGTGTCTTCAAATGT	GCTCTCCTCTGCCTGTGTCT	206	(GA)9
G02709	ATG0AGCCTGCTTCTCCTCT	TCAGCTATAAATTCAACTGGCTTA	151	(CT)14(GT)(CT)2
G02710	G0CACGTTAGTCTAGTTCTCTG	TAATCAGGTTCTTGGAA0AT0AC	139	(QA)8AT(GA)
G02712	CCAAATTCAG0ATTTCTGACTCC	ATG0AGCCTGCTTCTCCTCT	161	(QA)12
G02806	GCAGCCAATATGACATCATCC	TACAT0GAGCCTGCTTCT0CC	161	(QA)8
G02807	TGCATGGAGCCTGCTTCT0CC	0AAC0CTTTTGCAGCA0CC	175	(CT)11
G02812	TAGCTGT0AGCCT0GGTGT00A	00CACTTCACTTAATCTTT0AGT	114	(CT)7
G02813	CGAGGAT0GAAT0CCAC0TC	TCATTTGTCACTTATTA0T0CAC	174	(CT)2GC(CT)9(GT)3
G02814	TGCTGCTTTATAGTAAAAATG	CCTGCTTCTCCTCTGCTAT	265	(CA)5(GA)12
G02815	TCCTGCTGAATATGACGTTCA	AAGGGAGGG0AAACGACACAT	154	(CT)15
G02817	ATCGAATCC0CATCAG0CTC	CACAAAT0TAAACTG0GTATATT	177	(CT)9
G02819	ACACTCAGCATAGAGTCT0CTTG	CACCAGGTTG0AAAT0AATAAG	154	(CT)13
G02821	CCTGCACAGAGCCTGCTTCTC	AA0CACTGAGCCACCC0GACT	153	(CT)14
G02902	GATTGAGTCC0CATCAG0CT	AGCTGT0TTTAT0ACTACACATG	241	(CT)2TG(CT)6...(CT)6
G02903	TAGAGCCTGCTTCTCCTCTG	CCAATTTGAAGGATTCATCATT	146	(CT)2GT(CT)7GTAT(CT)8
G03001	TCCATCT0CCTATCACACCACT	TGAGCACTG0ATGTTATATGCAA	199	(TC)9
G03006	ATCTAATCC0CATT0GGCTC	AT0GGGAGTCA0CAGACCAGG	171	(TC)13
G03011	TAGGCTTCT0CTCAAGACAG	G0AT0GAGGAGAGCCTT0TTA	209	(GAT)6...(TC)9
G03012	CTGCTCTCTTTT0GCTCACTC	TTCTCCTCTGCTGTGTCT	141	(QA)17
G03013	ACTGAGATGG0AAGGG0CAGA	CTACATCGGCTCTAT0CTC	83	(QA)8
G03016	0AGCCTGCTTCTCCTCTGC	AGTCTGTGATTAGTTCTCAGAC	106	(CT)10
G03017	TCCTCC0A0ATTCTACAAT0AA		134	(QA)3CA(GA)9
G03018	TGCTTCTCCTCTGCTGTGT	CCTTCTGGATCTGCTTTTACTAT	203	(CT)13
G03019	0CACTCAGATGT0CCTATACTAT	AAACAGGATCGAGTCC0ACA	212	(QA)13
G03104	TAGCAGACAAAC0CCA0CTG	GAGCCTGCTTCTCCTCTG	167	(QA)13
G03109	CTGCATGGAGCCTGCTTCTT	TCTTATTCAAATCCTCCTGATTAT	153	(CT)9
G03111	CCTGCATGGAGACTGCTTCT	TGTTTCTCACTTCTTACT0A	218	(CT)21
G03601	GACACCAGGTTGATTATCATT	TGGAGACCTGGGATTGAGTC	166	(QA)10
G03901	ATCACACCTTGGGCTGAAGG	TG0AGCCTGCTTCTCCTCTG	174	(GA)14
G04801	AGGATGCCAGTTACATTT0AA	TGATGTTT0ATGTTACGTTGAT	208	(QA)18
G05002	CACGTGTATGTCTCTTATTAAG	CAGAGGTCTACTTTCTTCTG	170	(GA)30
G05602	CACATAACCACTGAACAACCT	GTCCACGTCAGGCTCTCTG	158	(QA)9
G05602	CACATAACCACTGAACCACCT	GTCCACGTCAGGCTCTCTG	158	(QA)9
G05604	TGCATGGGGCCTGCTTCTC	CCTCTTCATACCTCAGCAAGTG	169	(GT)9
G06202	CCCTTCTCTGTCTTTGAGAGT	AGCCTGCTTCTCCTCTGCC	144	(GA)3(CAG)9Q(GA)5
G06204	CTTCTCCTCTGACTGTGTCT	TCCTCAAAATTC AACATACAA	168	(CT)11(GA)3(CT)2
G06208	CCTGCTTCTCCTCCTCTG	TCCACAAAGCTCCTACTCAT	163	(CT)10
G06211	CCTGGGCGTGTAACCTGCT	CTGAAATGTAAAGTCAAA0G0AA	172	(CT)12...(A3C)8
G06219	CTAATATCAAAAGGTTATCCAC	CATCTTCTCTGCCAGT0TC	267	(QA)11
G06221	GGATAACCAGGATAATTTCTAC	AGAGAGGGCCACATCAG0CT	156	(AT)4(AT)3(GA)13
G06222	CTGCTTCTCCTCTGCTCT	AATTTATGGAAATGTTCC0AA	150	(CT)17(TC)3
G06224	0AGCCTGCTTCTCCTCTGCC	ACCCATGTATGAGCC0ATT0A	137	(CT)19
G06303	CAGGTGCTGCAAGAGCTTAGA	CTTCTCCTTTTCTCCTCTGCC	176	(QA)17
G06305	GTACAGTCTTCAACCTTCT	ATTGAGTCC0CCATCAGGCTT	215	(QA)14
G06316	AGCCTGCTTCTCCTCTCTC	CCACA0CTCAC00GT0TA	125	(CT)15
G06320	ACTGGCAATGGGTCT0AAAATAG	CTCAGTTATTTGTGGGCTCTTT	216	(QA)13
G06401	TGCTTCTCCTCTGTGTATCTC	CAGGTCC0CCTACACTAAGTG	133	(QA)10
G06402	ATGAATAGCTTGTGCATCAGTGCA	TGCTTCTCCTCTGCTGTGT	132	(QA)13
G06407	CCATCAAACCTTTACAGT0AA	GGGTCTGCTTCTCCTCTCT	163	(GA)12
G06407	CAATCAAACCTTTACAGT0AA	GGGTCTGCTTCTCCTCTCT	163	(QA)12
G06502	GTTAGGCTCTCTGTTCAGT0G	C00GTATACCTTCTCATCAT	146	(CT)9
G06601	TGTG0AACTGCTTACAATTTTC	T0CGTTACCTTACAAAGTTATTG	158	(CT)17
G06602	TGGAATCC0AGT0GGGCT	AT0TTACAAT0ATCTGATTATTCT	236	(CT)5GT(TC)12
G06603	CATTCAGATGCGGAGTTTC	CCAGGT0AGGTCAGGTT0TG	211	(QA)9
G06607	CTTCACAAGGTTGCACAAGAG	CTGCTTCTCCTCTGCTGT	159	(QA)14
G06608	TGATAGGACACTAGCAAGGCT	0AGCCTGCTTCTCCTCTCTC	194	(CA)2(QA)12
G06619	ACAACCTACAGAAAT0GAG0AA	CTTCCACAGCCTTTATTGT	196	(CT)10
G06701	GCTTTTAC0CAAC0ACTTAGA	AACCTGTGGCTCAGCAAGG	211	(QA)12
G06703	CTTCTCCTCTGCTGTGTCT	CGCTCTATAATCATCAGAAAT	159	(CT)11(GT)4
G06705	CTCTGCCCTGTGTCTGCTCTC	CTATACACATTGAGAAATGGCA	168	(TC)13

Table 2A (cont.)

G06706	ATCGAGTCCACGTCAGGCT	TTATTTATTTATTCATAGAGATGCA	98	(CT)13_ATG(A2T YA3T)2(A5T)
G06707	GOTGCATGGAGCCTGCTTCT	TGCCAGTTCAGTTTTCAAAGTT	147	(CT)17(GT)2
G06710	TTCTTTGTTTCTATTCTCTC	AACCCGGGATTGAGTCTG	167	(GA)14
G06713	GAGATCGAGTCCCATGTCAQ	CTTTGAGGAGATAAATCTTTCTA	225	(TC)20
G06714	ATCAAAATCCACATCGGGCTC	ATTAAGTTCAAACCTCCCAATG	163	(TC)12
G06715	TTGATCGAGTCTACATCGG	TCTTGGGTAAACTACTTAACTT	174	(TC)11
G06717	TGGAGCCTGCTTCTCCCTCT	CCTTATTCAGATTACCTGTTTG	147	(TC)8(TO)3
G06801	AGGAGCTGCTTCTCTCTG	CAATGATTATGGTTGTCAACTT	162	(CT)17
G06805	GACACCAACCGCTGAGCAC	GAGCTGCTTCTCCCTCTGCC	168	(CA)3(GA)9
G06901	GGCAGCTTTGATGACTGATTGA	AGTCTGTGTCAAGGCTCCCT	211	(GA)17
G06908	GGAAACAGTTAATTCATAAAATGAT	ATGGAGTCCCAAGTCAGGCTAC	205	(GA)3CA(GA)10
G06909	GGCAGCACTAAACCACTGAG	TGCTTTGCATCTTCCATTTT	209	(GA)15
G06910	CTGTGCTCAGCGGGAGTCT	TTATCTTAGAGTGAATGGAGATGG	101	(CT)15
G06914	GGAAAGATGTTGTCTCTTATCA	GGGTAGGGGTTTTGTTTATGG	159	(GA)20
G07001	CTACATGGAGCCTGCTTCTCC	TCCCCACAACTTTATGTCTC	129	(CT)11(GT)4
G07002	CCTTCTCCCTCTGCCCTGTG	GGCACTGATTATTTCTCTGTA	197	(CT)11
G07004	TGCTTGCTCTCTCTCAAATAA	GTGCATGGAGCCTGCTTCT	181	(GA)10G(GA)3
G07005	CCCTCTGCCTGTGTATGTGTC	ATGCCAGCAGGGAGTAGTCA	134	(CT)12
G07006	CAGTGGGGAATCTGCTTGAQ	CATTTCACTACATATACAGGTGTCA	150	(CT)11.....(CT)10
G07007	AATACCTGGGTAACAATTTA	GGATCGAGTCCCATGTC	156	(GA)11
G07008	GTGCATGGAGCCTGCTTCTC	AATGTACCTGTCCCTTTTG	127	(CT)13
G07301	GCATTCACCAATAGTCTTG	GCTGCTTCTCCCTCTGCCTAC	135	(GA)13
G07308	TCTCAATTTGAAAAGTTATAATC	TTCTCCCTCTCCCTCTATC	174	(TA)3.....(GA)5..... (GA)7
G07310	TATGCTTCTCCCTCTTCTCTG	GGTTTCTCTCCTTGATTTGTAAG	159	(CT)14
G07312	CTTCTCCCTCTGCCTGTGTC	TGCTAAACTCAACTCTCCTAA	123	(CT)14
G07314	CCATCAGTTTGTCTCTATCA	GAAGCTAAGTGAGGAGTAG	224	(CT)11
G07402	GGAGCCTGCTTCTCCCTCTG	TATCGTGCCCACTGCTGAAT	244	(CT)11T2(CT)5
G07406	AATTTAGTGAAGAAATGAAAGATG	GAAATAGCCTTAAAGCAATGTA	221	(GA)14
G07407	CCACCTGGGCTGCACTGAAGA	TGGAGCCTGCTTCTCCCTCTG	134	(GA)10
G07408	TGTCACTTGTCTCCACCTG	AGTGCTAAAGTTCTTCTATTG	135	(CT)14
G07410	ATCTCTTCTGCACTCCTGCT	CACGTAAAGGATGAGTTCAGGT	147	(TC)8(TO)2
G07413	CTGGAACAGAACCCACAATA	ACGAGATCAGTCCCACTCAG	231	(GA)24
G07414	TCCCTGAAAGGGGCATTAAAGACC	AGCCTGCTTCTCCCTCTGCCTATG	128	(GA)11
G07420	TCAGGAGGTGAGTTGCTTGGAG	CGGTGCATGGAGCCTGCTTCT	162	(CA)3(GA)16
G07502	CTCCCTCTGCCTATGTCTCTG	ACAGCCCTGTTTACCGAGGTG	255	(CT)14
G07503	CAGGAAACTGCTGGACTTGTGCT	TGCTTCTCCCTCTGCCTGTGT	126	(GA)15
G07504	AGTTCTGGAGCTGGGAAGTC	GGTGTAAGTGGCTCTTAGATA	215	(CT)23
G07505	TGCATGGAGCCTGCTTCTC	AGCAGTTACTCTTAGTGACTCC	138	(CT)11
G07506	ACTTCTCCCTCTGCCTGTGT	TTCCAGTGTATGTTGATTGAA	124	(CT)13
G07507	ATGGAGCCTGCTTCTCCCTCT	GTTTCTGCTCTCCTACCTGG	163	(TC)9
G07508	AGCCCTGCTTCTCCCTCT	GATTTGATTACATTCAAGTACA	98	(TC)10
G07510	AGGCATCCCTTACTTACTTACTTG	TCCACATCAGGCTTGCTGTAT	152	(GA)9
G07701	TATTCAGCCATTGACGGATTG	CATGGAGCCTGCTTCTCCCTC	247	(TC)2(TC)2GCC(T C)16
G07703	CTGCTTCTCCCTCTGCCTATG	TTTCCAACATTATGCTATGAT	198	(CT)14
G07704	AGCCTGCCCTCTCCCTCTCCA	AGAOTCACAATGCAACCCACAA	246	(TC)24
G07706	GGTGACACTATACTGAACCTTCT	TCTTCTCCCTCTCCCTCTGA	116	(GT)11
G07707	CTCCCTCTGCCTGTGTCTCTG	AATTTTATGTGCTCGGTCAOCC	202	(CT)9
G07709	CATTTGCTCATGTGCTGACTGA	CATGGAGCCTGCTTCTCCCTCTCC	147	(GA)16
G07710	GCTTCTCCCTCTGCCTCTATCTCT	ATTGATCCCGGATTTTGGTAATA	175	(CT)9
G07711	TAGTTCTTCTGCOCTTCTCC	CATTTCCAATCCATTAGAQA	149	(CT)9
G07712	CTGCATGGAGCCTGCTTCTC	TCAGACGCTCAACCAACTGAG	179	(CT)9
G07713	CTTGAAGCGCGGTGTTCTTG	TTGGACTTCTCTCCCTCTCCT	234	(GA)16
G07803	CAGCATGGAGTCTGCTTGTG	AGCTAAACATTAAACCAACTGAG	219	(CT)14
G07804	GGGTAGAACTGACATTCTTT	CTGTAGGGAGCCTGCTTCTC	133	(GA)7CA(GA)8
G08002	GGTATGGTCTGGAGACCTG	CTAATTOAGGAGATAGGATACATAA A	153	(CT)17
G08003	GTCAGCTTAGCCATTGAAGAAT	CCTGCTTCTCCCTCTGCCCTC	174	(CA)2(GA)15
G08003	GTCAGCTTAGCCATTGAAGAAT	CCTGCTTCTCCCTCTGCCCTC	174	(CA)2(GA)15
G08004	GGCACAACACTCTGAATTATTAG	CATCATTTATTGCCCTACTTTTA	175	(GA)15
G08005	GGTCTTCACTGCAAGGGAACCT	CATCAGATACTCCAACATTGAG	190	(GA)20
G08007	CAGAGTATCCTTGCCCTGTAG	GTGCCCTGGAGCCTGCTTCT	139	(CA)3(GA)12
G09201	TGGTACTGTAGCTTTGAAGAT	TCTGTGAAAGACACCTATTTA	173	(CT)14

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Table 2A (cont.)

H03501	TTGCCTTCTGGGTGTATTGACTT	GAATGTGGTTAGTAGAATTATACAG	300	(AT3)10(AT2)2AT
H03502	GATCCTGATIGTTCTTGAG	GGCATGGAGCATACTTCA	135	(AT3)4
H06601	TGCTTCTCCCTCTCCCTGT	TGGTGAAAGATTAGCCTGTGGA	125	(AT3)5(AT4)A T3)2
H06602	AAGTCCACATCAGGCTC	ACGTCAACCACAACCATCTAA	165	(AT3)12
H09205	CATTGCTGAGTCAAGGAATTCT	AGTTAOCCTGGAAGTTGTCAGAA	200	(AT3)12
H08505	TGCATGGAGCCTGCTTCT	CTTCTACACATGTTGTCCCT	160	(AT6)X(AT4)2(AT3) 13
H09208	AGTCCAGCATCACCGTTTGT	GAGGCTTATTTTCTGTCCAGTT	144	(AT3)9(AT4)
H10101	TCAGGCTCATGGGATTGAAGCTTC	TGCCATTGCACAGGATATAGGTCCA	305	(AT3)11
H10103	TCCACACTCAGTGCAGAATCTGCTT	TGTGAGACCGCAGAATACAGTACTC	141	(AT3)11

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Amplification reactions were carried out under standard PCR conditions described above using the annealing temperature indicated for each locus or a touchdown PCR protocol (Don, R.H. et al., *Nucleic Acids Res.* 19:4008 (1991)) was established. The variability of these loci were evaluated using the dog panel. For each locus, 5-10 dogs were studied in each breed. The number of alleles observed are presented in Tables 3A and 3B.

Table 3A

Marker Locus	Mixed Breed	Cocker Spaniel	Labrador Retriever	German Shepherd	Beagle
D00101	3	2	2	2	3
D00401	5	4	3	6	4
D01205	4	2	4	4	4
D01902	6	4	6	3	4
D02001	4	3	3	2	4
D02005	3	3	3	3	3
D02011	3	1	3	3	2
D02012	5	4	3	3	4
D02202	4	1	2	3	4
D03709	5	4	3	4	2
D03805	6	4	4	3	3
D03908	4	4	3	5	4
D04403	2	3	1	1	3
D04702	3	1	3	2	3

Table 3B

	Marker Locus	Doberman Pinscher	Siberian Husky	Scottish Terrier	English Pointer	Greyhound
5	D00101	3	2	2	3	2
	D00401	3	6	5	5	5
	D01205	2	2	1	3	3
	D01902	5	3	4	4	7
	D02001	2	4	3	2	3
10	D02005	1	3	2	3	3
	D02011	2	3	4	5	2
	D02012	3	3	4	4	3
	D02202	1	3	2	2	1
	D03709	4	6	4	5	4
15	D03805	3	7	4	5	4
	D03908	3	8	3	4	4
	D04403	1	3	2	3	3
	D04702	2	3	2	3	2

In general, all of the microsatellite loci tested displayed variability within and across breeds. While 9 cells out of 140 (6.4%) in Tables 3A and 3B were monomorphic, these were scattered though 6 different microsatellite loci, which were quite polymorphic in other breeds. The maximum number of alleles detectable by this analysis for a locus in a given breed was 8, in the case of locus D3908 in the Siberian Husky. The percent heterozygosity observed at each locus in each breed is presented in Tables 4A and 4B.

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Table 4A

Marker Locus	Mixed Breed	Cocker Spaniel	Labrador Retriever	German Shepherd	Beagle
D00101	20	0	0	0	90
D00401	100	100	100	88	25
D01205	70	50	0	22	64
D01902	100	100	100	11	36
D02001	40	86	57	50	33
D02005	90	29	38	22	27
D02011	38	0	25	44	18
D02012	0	17	33	0	33
D02202	20	0	0	0	0
D03709	20	100	75	89	50
D03805	100	50	50	30	67
D03908	100	100	100	88	100
D04403	100	100	100	100	100
D04702	22	0	80	0	30

Table 4B

	Marker Locus	Doberman Pinscher	Siberian Husky	Scottish Terrier	English Pointer	Greyhound
5	D00101	60	0	78	86	38
	D00401	33	50	86	67	100
	D01205	60	44	0	86	25
	D01902	100	63	100	100	100
	D02001	100	57	25	50	13
10	D02005	0	50	77	71	100
	D02011	20	33	44	43	50
	D02012	0	50	17	40	0
	D02202	0	0	17	17	0
	D03709	100	78	100	86	100
15	D03805	100	67	100	80	29
	D03908	33	44	100	100	100
	D04403	30	50	56	14	29
	D04702	67	20	33	60	40

No heterozygotes were observed in only 21 out of 140 (15%) of the loci/breed combinations studied. At the same time, 30 out of 140 (21%) cells showed 100% heterozygosity. The mean and standard deviation of heterozygosity observed for each locus across different breeds, as well as the mean and standard deviation of heterozygosity observed within each breed across different loci are shown in Figures 1A and 1B, respectively. The breeds studied show a mean heterozygosity ranging from 36 to 60% across different microsatellite loci with considerable standard deviations. Among the loci studied D03908, D01902, D03709 and D00401 showed the highest mean heterozygosity across breeds of 87, 81, 80 and 75%, respectively. The number of repeats in the reference clone in these loci were 16, 18, 12 and 22. The least informative loci across breeds were D02202 and D02012 at 5 and 19% mean heterozygosity, respectively. The number of repeats in the reference clone in these loci are 12 and 15, respectively. Correlation analysis did not reveal any significant linear relationship between the number of repeats at a locus and its overall observed heterozygosity ($r=0.22$).

Figures 2A-2D show the results from typical gels used to evaluate the alleles in gathering the data as described above. Amplification products of DNA from various different breeds at the locus D02011 are shown. Figures 2A-2D represent different gels, run under similar conditions. Note that the molecular weight marker identified in lanes marked M is the 246 bp band of the 123 bp ladder (Gibco-BRL, Gaithersburg, MD). The size of the amplification product in the reference clone was 238. The different alleles are easily identified, with PCR products separating in sharp and well resolved bands, near and below the 246 bp marker. Some non-specific amplification products can be observed, especially in cases with higher template DNA concentrations; however, these do not interfere with correct typing.

The results indicate that microsatellite loci containing CA repeats are abundant and highly polymorphic markers for the canine genome. These findings indicate that such markers hold great potential for use as linked markers for genetic defects in pure bred dogs.

The estimate that there is one useful CA repeat every 31 kb in the canine genome is in good agreement with one every 42 kb estimated recently by others (Rothuzien, J. et al., *Theor. App. Genet.* 89:403-406 (1994)). In the above-described study, a secondary screening was carried out and only very strong hybridization signals were accepted as positive, which resulted in elimination of about 20% of the primary positives. It thus appears that the estimate of the minimal CA microsatellites

frequency in the canine genome is accurate. These estimates have practical implications particularly, since most cosmids have insert sizes in the 30-40 kb range, the likelihood of finding a useful CA repeat in a cosmid clone harboring a gene of interest is high.

5

SPECIFIC EXAMPLE II

Materials and Methods

Patients and pedigrees. The patients and pedigrees used were primarily those used and described earlier (Yuzbasiyan-Gurkan, V. et al., *Genomics* 15:86-90 (1993)). Briefly, pedigrees of American Kennel Club registered Bedlington terriers were associated with the help of Bedlington terrier (BT) breeders. While all of the pedigrees have a family history of CT, not all had a symptomatic proband at the time of pedigree ascertainment. Diagnosis of dogs as to whether they were affected or unaffected with CT was made in all cases by quantitative copper assay from liver biopsies performed at 1 year of age or older by criteria earlier described. DNA was extracted from peripheral blood samples collected in acid-citrate-dextrose as anticoagulant as described (Yuzbasiyan-Gurkan, V. et al., *Genomics* 15:86-90 (1993)).

Microsatellite analysis. The microsatellite markers used in this study were developed as described in Specific Example I. Standard conditions used to amplify each marker locus in polymerase chain reactions (PCR) were as follows: 25-50 ng of genomic DNA as template in 25 μ l of PCR buffer (50 mM Tris HCl, pH 8.3 @ 25°C, 50 mM KCl, 1.5 mM MgCl₂), 200 μ M dNTPs, 200 pM with respect to each primer and 1.5 U of Taq DNA polymerase. A touchdown PCR protocol (Don, R.H. et al., *Nucleic Acids Res.* 19:4008 (1991)) was established to facilitate the robust amplification of most markers under the same conditions. PCR was carried out at 94°C for 45 sec., 52°C for 30 sec., and 72°C for 1 min.

The microsatellite markers were initially evaluated in ten sets of parents from the BT pedigrees. Those markers for which at least one parent was heterozygous were then evaluated in all the dogs in the pedigree. Seven to twelve microliters of product were run on a 5% to 7% Hydrolink D600 acrylamide horizontal gel according to the manufacturer's instructions with the following modification. During the overnight runs, a plexiglas gel carrier was placed on top of the gel to prevent the swelling and distortion that was otherwise observed. Initially, electrophoresis was carried out from 4 to 5 hr. at 50 V in 1 X TBE (90 mM Tris, pH 8.3, 90 mM boric acid, 2 mM EDTA) with ethidium bromide. A photograph was taken and the gel

electrophoresis then continued overnight at 35-40 volts depending on the fragment size of the product. A second photograph was taken and the results visually evaluated. It was found that two photographs were helpful in comparing different dogs with similar patterns. The alleles were then tabulated and used in linkage analysis.

Linkage analysis. Two point LOD (logarithm of odds) scores between CT and all the markers tested were generated using the MLINK program of the LINKAGE package (v5.1) (Lathrop, G.M. et al., *PNAS (USA)* 81:3443-3446 (1984)). A gene frequency of 0.5 was assumed for CT.

Results

Two hundred thirteen microsatellite markers were evaluated in the process of finding linkage. Of these 213 markers, 181 provided scorable products in BTs using the touchdown protocol described above. Of these, 114 were informative in the pedigrees and were further evaluated.

Of all the markers tested for linkage to CT, only one yielded a significant LOD score. As shown in Table 5 below, marker number C04107 was found to be linked to the CT locus at a LOD score of 5.96, at a recombination fraction of zero. No recombinants were detected. Since a LOD score of 5.96 indicates that the odds of observing this linkage by chance is about 1 in a million, and since, a LOD score of greater than 3 or an odds ratio of 1 in 1000 is considered proof of linkage, the findings imply that the CT locus is indeed very close to the C04107 locus and thus can be used to predict the inheritance of alleles at the CT locus. No recombinants were detected in this study and thus a value can not be put on the genetic distance between these loci, except to say that they are very close.

Table 5

θ (Recombination Fraction):	0.0	0.001	0.01	0.05	0.15	0.1	0.2	0.3
C04107 vs. CT	5.96	5.95	5.85	5.38	4.78	4.14	3.49	2.13
C04107 vs. ESD	$-\infty$	-19.73	-10.78	-4.77	-2.44	-1.28	-0.6	-0.01
C04107 vs. RB1	$-\infty$	-20.35	-11.43	-5.47	-3.18	-2.01	-1.28	-0.47

The primer sequence and allele information about this marker are shown in Table 6. The allele frequencies were determined from alleles observed in apparently unrelated dogs.

Table 6

5	Marker Locus	C04107
	Repeat Motif in Reference Clone	(CA) ₆ CT(CA) ₁₁
	Primer Pair	TCAGCAACTATACATTTAAGAGGA CTGTCCCATCTAAAGGATAGG
	Allele 1 and Frequency	163 bp, 0.39
	Allele 2 and Frequency	167 bp, 0.61

10 Marker C04107 was used to locate markers C04107B and C04107C shown in Table 2A, which are close to C04107 and also contain repeats. This "family" of markers may be used to detect CT.

A typical pedigree illustrating linkage to C04107 is shown in Figure 3. In Figure 3, circles and squares depict females and males, respectively, and individuals affected with CT are indicated by the filled symbols. The asterisk in the figure indicates an individual not available for analysis. The bands are the negative image of amplification products obtained from the dogs indicated in the pedigree and analyzed individuals share the 2,2 genotype at this locus. In this pedigree, all dogs with the 1,1 genotype are predicted to be homozygous normal while those with the 1,2 genotype are predicted to be heterozygous, and thus carriers of the CT gene.

Given the finding of linkage and allowing for a small error for recombination, it is predicted that all the offspring with the 1, 1 genotype are clear of the CT gene i.e., homozygous normal, and that all 1, 2 offspring are carriers in this pedigree.

Since data on the ESD and RB1 loci were available for most of the dogs from a previous study (Yuzbasiyan-Gurkan, V. et al., *Genomics* 15:86-90 (1993)), the linkage relationships of these loci with C04107 were also evaluated. Neither ESD or RB1 were found to closely linked to C04107 (see Table 5).

As demonstrated by the pedigree illustrated in Figure 3, given an informative mating, it is now possible to identify all the genotypes in the offspring, distinguishing between the homozygous normal, homozygous affected and heterozygous dogs provided the genotype of one affected dog is available. However, C04107 is not extremely polymorphic in the BT population, showing only two alleles and a

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calculated heterozygosity of 0.43. Therefore, typing at the C04107 will not always yield information about the CT status of the offspring. Thus far, all affected dogs have been of the 2,2 genotype and the 2 allele is more common than the 1 allele (see Table 6). The matings which produce affected dogs will be found to be either
5 between parents who are both 2,2 both 1,2 or one 1,2 and the other 2,2. In such cases, typing at the C04107 locus will only be useful in the second and third mating types. In the latter mating pairs, predictive information would only be available as to which dogs are affected. In order to make most pedigrees in the breed informative, additional polymorphic markers closely linked to C04107 are developed.
10 It is predicted that a battery of three to five highly polymorphic markers will make almost every pedigree informative.

If strong linkage disequilibrium occurs at C04107 or nearby loci, the predictive power will be substantially improved. However, further studies of allele distributions in the BT population are needed to evaluate linkage disequilibrium. In any case, it
15 should be possible to dramatically reduce the frequency of this serious disease within a very few generations.

As discussed above, canine copper toxicosis is present in the West Highland White Terrier and perhaps in several other breeds. (Thornburg, L.P. et al., *Vet. Pathol.* 27:81-88 (1990)). In the West Highland Terrier, it is clear that the phenotype
20 is more complex, in that there is a spectrum of liver copper levels. This marker is evaluated in the West Highland White Terrier breed and it is determined whether there is segregation of high liver copper values with C04107.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize
25 from such discussion and from the accompanying claims and drawings, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention.

All publications referred to herein are expressly incorporated by reference.

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WE CLAIM:

1. A primer comprising a polynucleotide, wherein the polynucleotide has a sequence selected from the group consisting of the sequences of Table 2A.
2. The primer of Claim 1, wherein the sequence is the Sns sequence of marker locus C04107 of Table 2A.
3. The primer of Claim 1, wherein the sequence is the Asn sequence of marker locus C04107 of Table 2A.
4. The primer of Claim 1, wherein the sequence is the Sns sequence of the marker locus C04107B of Table 2A.
5. The primer of Claim 1, wherein the sequence is the Asn sequence of the marker locus C04107B of Table 2A.
6. A method for amplifying DNA, comprising the step of performing PCR with the DNA and a primer set selected from the group consisting of the primer sets of Table 2A.
7. The method of Claim 6, wherein the primer set is that shown as the Sns sequence and Asn sequence of the marker locus C04107 of Table 2A.
8. The method of Claim 6, wherein the primer set is that shown as the Sns sequence and Asn sequence of the marker locus C04107B of Table 2A.

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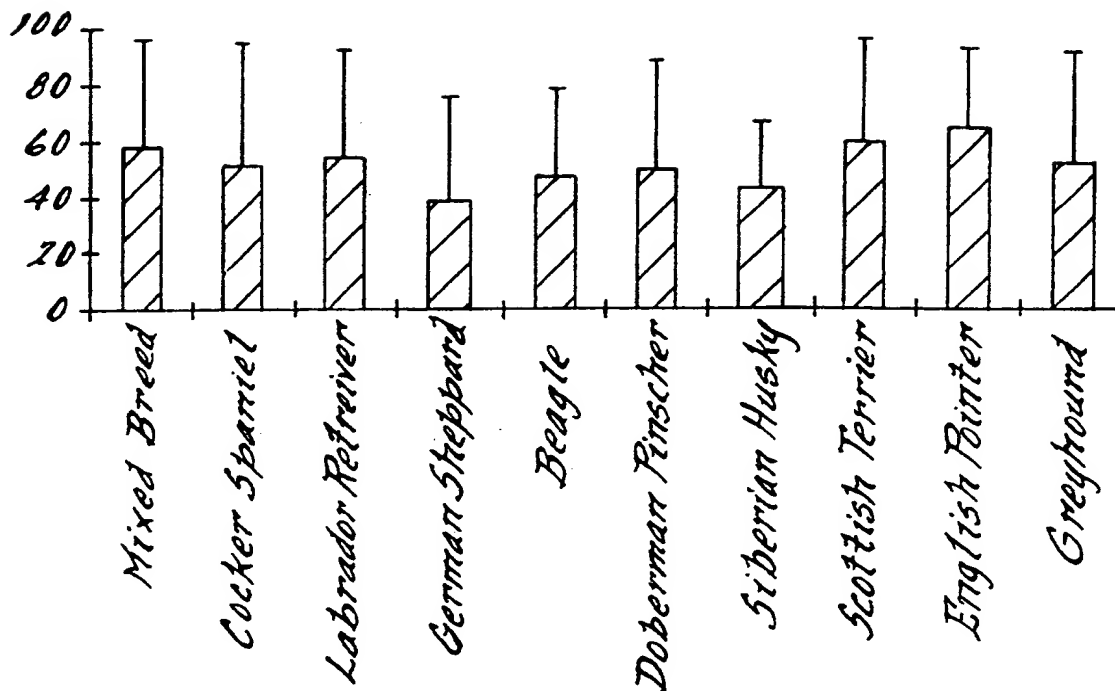


Figure 1A.

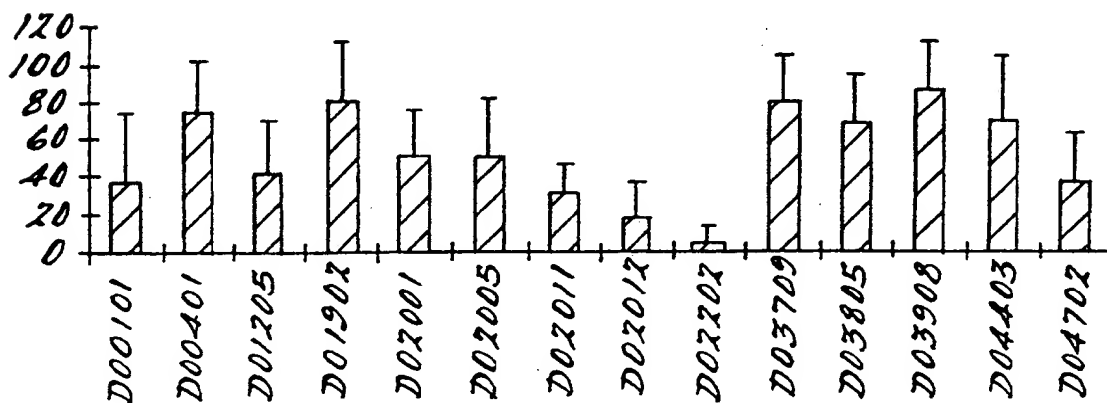
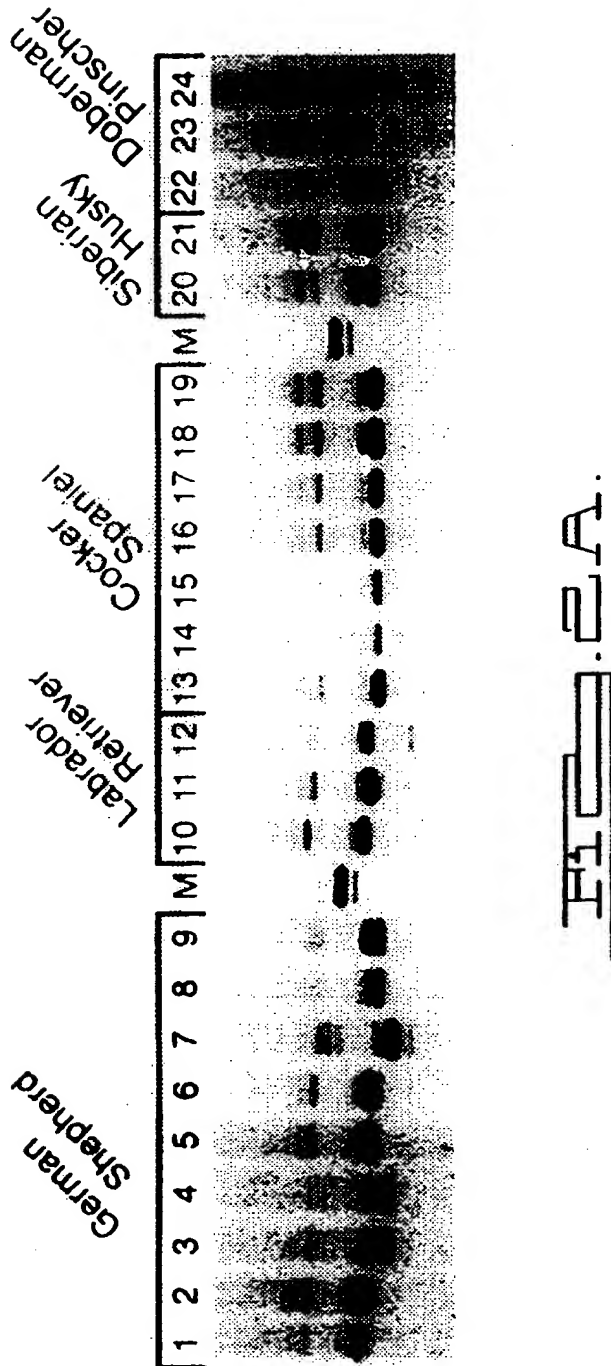


Figure 1B.

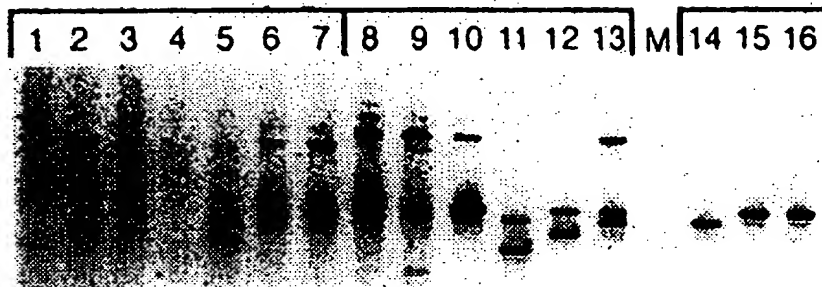


3/4

Pointer

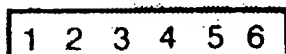
Scottish
Terrier

Scottish
Terrier

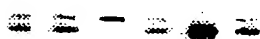


Fi 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466

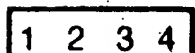
Greyhound



FIN. 2 C.



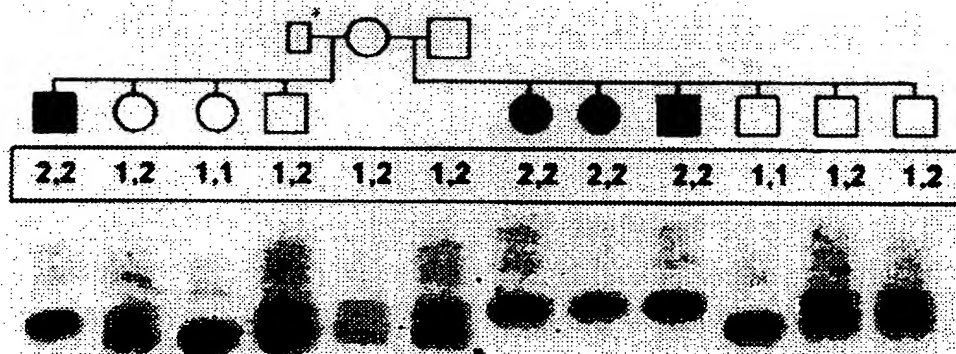
Beagle



FILED.



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FIG. 2.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/02396

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C12Q 1/68

US CL : 536/24.33; 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 24.33; 435/6. 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OSTRANDER et al. One hundred and one new simple sequence repeat-based markers for the canine genome. Mammalian Genome. March 1995. Vol. 6, No. 3, pages 192-195, especially abstract and Table 1.	1-8 (in part)
Y	OSTRANDER et al. Identification and Characterization of Dinucleotide Repeat (CA) _n Markers for Genetic Mapping in Dog. Genomics. April 1993. Vol. 16, No. 1, pages 207-213, especially Table 2.	1-8 (in part)
A	YUZHASIYAN-GURKAN et al. Linkage Studies of the Esterase D and Retinoblastoma Genes to Canine Copper Toxicosis: A Model for Wilson Disease. Genomics. January 1993. Vol. 15, No. 1, pages 86-90, especially page 86.	1-8 (in part)

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 JUNE 1997

Date of mailing of the international search report

08 JUL 1997

 Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
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DEBRA SHOEMAKER

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02396

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FREDHOLM et al. Efficient resolution of parentage in dogs by amplification of microsatellites. Animal Genetics. February 1996. Vol. 27, No. 1, pages 19-23, especially page 21.	1-8 (in part)
A	ROTHUIZEN et al. The incidence of mini- and micro-satellite repetitive DNA in the canine genome. Theoretical and Applied Genetics. October 1994. Vol. 89, No. 4, pages 403-406, especially pages 405-406.	1-8 (in part)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/02396

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8, as limited to 10 sequences

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/02396

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

searched for inventors and keywords: microsatellite or linkage or polymorphism or allele and dog/canine genome or gene or dna and ca repeat and copper toxicosis in APS, CAPLUS, MEDLINE, SCISEARCH, LIFESCI, EMBASE, BIOSIS WPIDS. Searched sequences of elected group by registry, genbank and dgene.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

each of the 519 microsatellite markers disclosed in Table 2A are distinct species. It is noted that in two cases there are more than one primer set corresponding to the same loci, for example C01407, C01407B and C01407C, which do have unity with each other.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 1 and 6 are generic to each of the 519 microsatellite markers disclosed. Claims 2-5 & 7-8 have unity with each other because a single microsatellite locus is claimed but do not have unity with claims 1 & 6 because distinct microsatellite loci are claimed.

The following claims are generic: 1 & 6.

Applicant is allowed to select 10 sequence for the search fee and pay an additional \$200 for each additional 4 sequences to be examined. Since there is unity of invention between C01407, C01407B and C01407C, these sequences are considered to be one species. A search report will be established on C01407, C01407B and C01407C and the first four primer pairs (so as to form a group of 10 sequences) recited in Table 2A if no other groups are paid for and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2, 13.2) for the reasons indicated below:

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each of the 519 microsatellite markers claimed in claims 1 & 6 are drawn to a unique nucleic acid sequence, each with a unique location in the canine genome and each linked with distinct genes and traits. Thus there is no special technical feature that relates to these microsatellite markers to each other.